Development of aggression and impulsivity

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Development of aggression and impulsivity: modulation by adolescent social stress?

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CHAPTER 1

General introduction

INTRODUCTION

Affective disorders have a major impact on the life of millions of people worldwide and are an important cause of disability. It is estimated that about 27% of the adult European population (age 18-65) is affected by at least one mental disorder in a period of 12 months (Wittchen and Jacobi, 2005).

Adverse social experiences early in life are well recognized predictors of the development of psychopathology later in life (Heim and Nemeroff, 2002; Gunnar and Quevedo, 2007; Danese et al., 2009; Nugent et al., 2011). Especially the development of internalizing (i.e. depressive and anxiety) disorders due to early life stress is well recognized (Edwards et al., 2003; Heim and Binder, 2012). In general, the prevalence of internalizing disorders is higher in women than in men (Hankin et al., 1998; Kendler et al., 2002; Wade et al., 2002; Wittchen and Jacobi, 2005; Bouma et al., 2008). While internalizing disorders are directed inward and are an indication of the emotional and psychological state of an individual, externalizing behaviors are displayed outwardly and are directed towards the physical environment (Liu et al., 2011). Exposure to early life stress can also lead to externalizing disorders, such as antisocial personality disorders and the expression of inappropriate levels of aggressive behavior. These disorders are, in contrast to depression, more often diagnosed in men (Kessler et al., 1993; Kessler et al., 1994; Nolen-Hoeksema, 2012).

Aggression is one of the most prevalent criteria in externalizing disorders (DSM-IV). Regulated aggressive behavior can transform into the display of uncontrolled violent forms of behavior. Violence can have serious consequences and is an expensive public health problem worldwide. The World report on violence and health launched by the World Health Organization (WHO) in 2002 estimated that each year, approximately 1.6 million people die as a direct result of injuries resulting from interpersonal violence. Many more people are injured and suffer from a range of physical, sexual and mental health problems as a consequence of social stress (Krug et al., 2002). Despite these large figures, it is important to realize that the majority of human social interactions do not escalate into violence. Evidently, in most individuals there are strong inhibitory factors that normally prevent the transition of aggression into violence. Among other factors, certain personality characteristics appear to be predictive for this transition. Attention problems and a lack of impulse control are such personality characteristics involved in the development of violent behavior (Farrington, 1998; Eklund and Klinteberg, 2003). These characteristics seem to be linked to deficiencies of prefrontal cortex functioning (Davidson et al., 2000; Bufkin and Luttrell, 2005).

During adolescence, the prefrontal cortex is undergoing major structural and functional changes by reduction and reorganization (pruning) of excessive neural connections (Kalsbeek et al., 1988; Giedd et al., 1999; Andersen et al., 2000; Spear, 2000; Andersen, 2003; Tseng and O'Donnell, 2007; Casey et al., 2008).

The development of the prefrontal cortex is accompanied by distinctive adolescent behavior. Adolescence is characterized by low impulse control and engagement in riskier behavior compared to adult individuals (Laviola et al., 2003; Andrzejewski et al., 2011).

This is for example displayed by the association between drug abuse and the onset of puberty and adolescence (Laviola et al., 1999; O'Malley and Johnston, 2002; Schneider, 2008).

Furthermore, this developmental period is characterized by heightened self-consciousness, increased importance and complexity of peer relationships and learning of social skills (Steinberg and Morris, 2001; Blakemore, 2008). The change in socio-environmental context and frequent confrontations with novel situations and emotions that occur during adolescence might lead to the experience of stress. These environmental factors may play an important role in the modulation of the prefrontal cortex in this period, which in turn might play a key role in the development of violent behavior.

The main aim of this thesis is to study individual differences in the consequences of adolescent social stress on externalizing behaviors (i.e. adult aggression and impulsivity) in adulthood and to test the hypothesis that the behavioral outcome is modulated by synaptic plasticity in the prefrontal cortex.

AGGRESSION AND VIOLENCE

Aggression is an essential symptom in several externalizing disorders (DSM-IV). Two subtypes of aggression have been identified in humans. The instrumental aggressive subtype is highly controlled and purposeful. Reactive aggression on the other hand is more impulsive, more situational and unpredictable (Vitiello and Stoff, 1997). It is hard to find a true equivalent of instrumental aggression in animals mainly because its premeditated, purposeful character cannot be assessed objectively. Reactive aggression, as observed in humans, seems to be comparable to offensive aggression in animals.

In the animal kingdom, aggression is an important aspect of social behavior and is an essential characteristic in animal personality research. It has an important function in acquiring and defending a territory, to maintain social status and it is necessary to obtain vital resources such as food, water and shelter (Benus et al., 1991). Although aggression is a highly adaptive form of social behavior, it is potentially dangerous, may also lead to considerable harm and can have maladaptive consequences. For that reason, aggressive behavior is under strong inhibitory control by the brain to prevent its adverse effects (De Waal, 2000). For example, threatening behavior signals physical strength and aggressive intentions. It allows the chance of withdrawal and thereby may prevent physical attacks. Furthermore, ritualization may replace the actual aggressive response. The display of submissive behavior by the intruder also leads to strong inhibition of further aggressive behavior of the resident (Blanchard and Blanchard, 1977; Koolhaas et al., 1980; Haller and Kruk, 2006). Hence, aggression can be considered as an effective form of social communication.

Unregulated aggressive behavior can transform into the display of violent behavior. Violence can be defined as escalated aggressive behavior that is expressed out of context and out of inhibitory control, and does not to serve an adaptive function in social communication (De Boer et al., 2009a; Natarajan and Caramaschi, 2010).

It is unclear which intrinsic and environmental modulating factors are involved in the transition of aggression into violence. In this thesis I will focus on the role of impulsivity as an important factor in the transition of aggression into violence.

IMPULSIVITY

The initiation of aggressive behavior is closely related to impulsivity, as a trait-characteristic, both in humans and in animals (Cervantes and Delville, 2007; Rudebeck et al., 2007; García-Forero et al., 2009). Impulsivity is an important component of several externalizing disorders, such as attention deficit/hyperactivity disorder (ADHD) (DSM-IV) and antisocial personality disorder (Swann et al., 2009). In hamsters, high levels of impulsivity are related to high levels of aggression, whereas low aggressive hamsters are less impulsive (David et al., 2004; Cervantes and Delville, 2007).

Impulsivity or behavioral inhibition plays an important role in many types of behavior and is an aspect of behavior that can have both beneficial and detrimental outcomes. Fast decisions can result in better outcomes, for example due to saving of time in dangerous situations. However, impulsivity can also have negative consequences, i.e. a correct short term decision is not necessarily beneficial in the long run as well. Impulsivity is an important aspect of several psychiatric disorders and is among others associated with ADHD, eating disorders, mania, substance abuse and personality disorders (DSM IV, 1994).

The most common definition of impulsivity is action without forethought. However, impulsivity is not a unitary construct and consists of several heterogeneous categories of behaviors. The definition of these categories is a matter of debate among psychologists. However, the most common categories include decreased inhibitory control, intolerance of delay to rewards and quick decision-making due to lack of reflection (Winstanley et al., 2006a). These behavioral categories are reflected in distinct neuroanatomical as well as the neurochemical substrates (Evenden, 1999; Pattij and Vanderschuren, 2008). This is shown in figure 1.1, which summarizes the results of an extensive lesion study by Pattij and Vanderschuren (2008). Anatomically, the striatum, limbic brain regions and the prefrontal cortex have been shown to play an important role in aspects of impulsive behavior. Brain serotonin (5-hydroxytryptamine, 5-HT), dopamine, and noradrenalin systems have been shown to be involved in impulsive behavior as well (for reviews see (Cardinal, 2006; Dalley et al., 2008; Pattij and Vanderschuren, 2008)).

In this thesis I focus on the prefrontal cortex because of its key role in impulsivity (behavioral flexibility and behavioral inhibition), the involvement in aggressive behavior and the structural changes taking place in this brain area during adolescence.

PREFRONTAL CORTEX

The prefrontal cortex has been associated with both aggressive behavior (Blair, 2004; Siever, 2008) and various aspects of impulsive behavior (Mobini et al., 2002; Winstanley

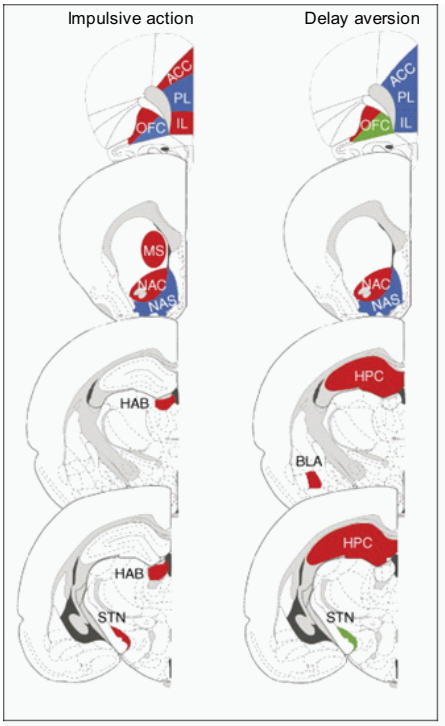

Figure 1.1 Schematic overview of the neuroanatomical regions in the brain involved in impulsive action (inhibitory control processes) and delay aversion. Red indicates that lesions of these regions increase impulsive action or delay aversion. Green indicates beneficial effects of lesions on impulsivity and on choice behavior. Blue indicates that lesions of these brain regions do not affect impulsive action nor delay aversion. Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; HAB, habenula; HPC, hippocampus; IL, infralimbic cortex; MS, medial striatum; NAC, nucleus accumbens core; NAS, nucleus accumbens shell; OFC, orbitofrontal cortex; PL, prelimbic cortex; STN, subthalamic nucleus (Pattij and Vanderschuren, 2008).

et al., 2006a; Dalley et al., 2008). The most well-known example of the link between aggression and impulsivity and the involvement of the prefrontal cortex is Phineas Gage. His prefrontal cortex became damaged during a railway construction accident, which resulted in the change into an irritable and hostile personality (Damasio et al., 1994). In general, patients with lesions of the ventromedial prefrontal cortex show more aggressive and violent behavior (Grafman et al., 1996).

In prisoners convicted for murder, the glucose metabolism in the prefrontal cortex measured by positron emission tomography (PET) is significantly lower compared to imprisoned controls (Raine et al., 1994). Studies using functional MRI have shown that several regions of the prefrontal cortex are more generally involved in decision making, tested with the Iowa gambling task, in humans (Lawrence et al., 2009). Performance on the Iowa card task improves between adolescence and adulthood (Overman et al., 2004). This improved decision making seems to be dependent on the maturation of the prefrontal cortex between adolescence and adulthood.

The prefrontal cortex is a late developing part of the brain; it is undergoing major structural and functional changes during adolescence (Kalsbeek et al., 1988; Giedd et al., 1999; Andersen et al., 2000; Spear, 2000; Andersen, 2003; Tseng and O'Donnell, 2007). For example, the density of prefrontal cortex derived axon terminals decreases significantly between adolescence and adulthood (Cressman et al., 2010). A pre-adolescent increase in cortical gray matter is followed by a post-adolescent decrease (Giedd et al., 1999; Insel,

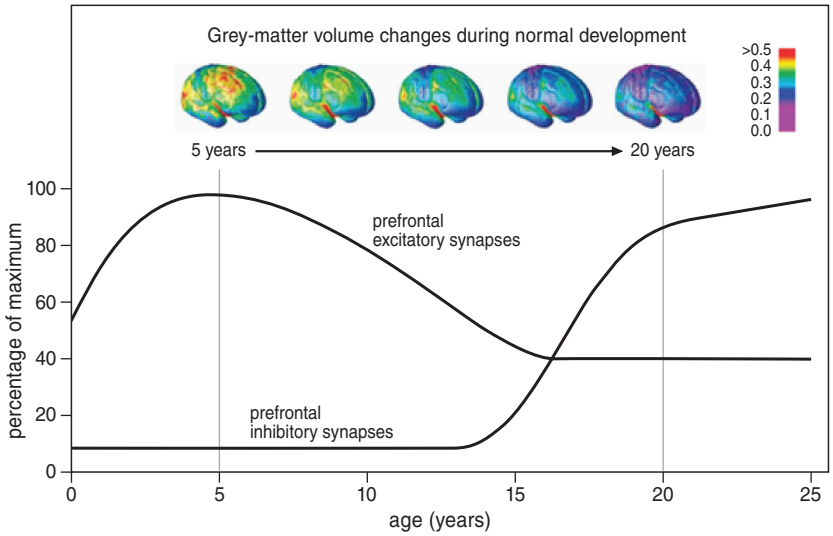

Figure 1.2 A progressive reduction in grey-matter volume takes place during adolescence. Data from human and non-human primate brain indicate increases in inhibitory and decreases in excitatory synaptic strength occurring in the prefrontal cortex throughout adolescence and early adulthood (adapted from: Insel, 2010).

2010) (figure 1.2). Also, dopamine D_1 and D_2 receptors are overproduced prior to puberty and pruned back to adult levels thereafter in the prefrontal cortex (Andersen et al., 2000). In view of the role of the prefrontal cortex in decision making and the profound neuroanatomical changes during adolescence it is conceivable that environmental factors during adolescence will have a major impact on prefrontal cortex maturation and hence on adult decision making processes. Therefore, my thesis focuses especially on this neuroanatomical area.

SYNAPTIC PLASTICITY

Synaptic plasticity is defined as an experience-dependent change in the strength of synaptic connections between neurons. In recent years, perturbations in synaptic plasticity have been connected to major depression and are thought to play an important role in pathogenesis of diseases across the central nervous system. Defective synaptic plasticity in the prefrontal cortex is linked to attention-deficit and impulse-control disorders, for example (Dickstein et al., 2006; Brennan and Arnsten, 2008). Hence, it is important to understand how the social environment during adolescence may lead to long-lasting changes in synaptic connectivity.

Synaptic plasticity is among others regulated by neurotrophic factors (Calamandrei and Alleva, 1995). The focus in this thesis is on the neurotrophic factor BDNF (Brain-derived neurotrophic factor). Although neurotrophic factors themselves do not affect

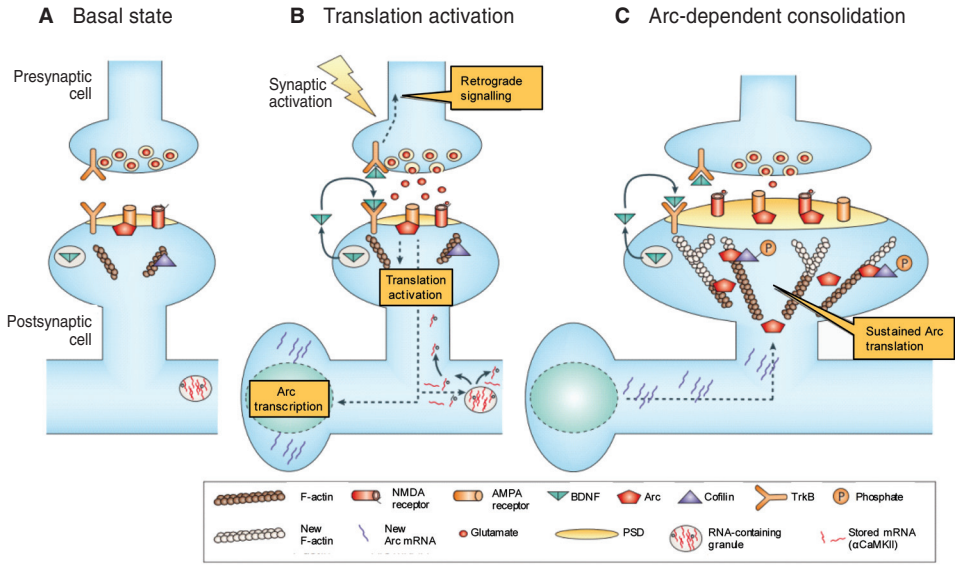

Figure 1.3 A model for Arc-dependent LTP consolidation in the dentate gyrus. (A) Neuron in the resting (basal) state. (B) Translation activation. (C) Arc-dependent consolidation. Abbreviations: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid; BDNF, brain-derived neurotrophic factor; Arc, activity-regulated cytoskeleton-associated protein; TrkB, tropomyosin-related kinase B; PSD, postsynaptic density; αCaMKII, α-subunit of calcium/calmodulin-dependent protein kinase II (Bramham and Wells, 2007).

mood, they determine the plasticity of synaptic networks involved in mood regulation (Duman and Monteggia, 2006). BDNF has emerged as a major regulator of synaptic transmission and plasticity at adult excitatory synapses (Bramham and Messaoudi, 2005). It regulates experience dependent changes in synaptic connectivity, is implicated in stress-induced or reactive depression and the therapeutic effects of antidepressants (Castren and Rantamaki, 2010).

BDNF appears to be one of the major activity-dependent modulators of dendritic proteins synthesis (Bramham and Messaoudi, 2005). It is involved in both the induction and consolidation of long-term potentiation (LTP). BDNF is synthesized, stored and released from glutamatergic neurons (Lessmann et al., 2003).

High-frequency stimulation causes the activation of postsynaptic NMDA (*N*-methyl-D-aspartate) receptors and BDNF release from or near glutamatergic synapses. BDNF binds to the postsynaptic TrkB (tropomyosin-related kinase B) receptor which leads to rapid post-synaptic translation activation and induction of the immediate early gene Arc (activity-regulated cytoskeleton associated protein, also known as activity-regulated gene 3.1 (Arg3.1)) transcription in the nucleus (figure 1.3B) (Link et al., 1995).

In Arc-dependent consolidation, Arc mRNA is transported to dendrites and locally captured. Sustained translation of dendritically transported Arc is necessary for cofilin phosphorylation and local F-actin expansion. F-actin formation promotes the growth of

the postsynaptic density and the spine head and the formation of stable LTP (figure 1.3C) (Bramham and Wells, 2007).

Because BDNF and Arc are key-players in synaptic plasticity, they will be used in the present thesis as molecular markers of synaptic plasticity.

ADOLESCENCE

Besides developmental changes in the brain, adolescence is a period of life that is characterized by major biological, psychological and social changes. It is the period in which childhood temperament develops into adult personality. Behaviorally, adolescence is characterized by risk taking and impulsive behaviors which reflects an immature prefrontal cortex functioning (Laviola et al., 2003; Kelley et al., 2004; Andrzejewski et al., 2011).

Furthermore, adolescence is considered as a particularly vulnerable period for the development of adult mental disorders. Mental disorders in adulthood cause much suffering, disability, loss of quality of life and pose a costly burden on the Western society. It is estimated that 21% of the total burden of disease in the Western world is due to mental disorders (Murray and Lopez, 1997). The majority of chronic-recurrent adult mental disorders are first expressed during adolescence. From age 15, incident rates steeply rise for anxiety disorders (Bernstein et al., 1996), substance abuse and dependence (McNeill, 1991; O'Malley and Johnston, 2002; Schneider, 2008) as well as delinquency (Landsheer and `t Hart, 1999) and depressive disorders (Hankin et al., 1998). As an example, figure 1.4 illustrates the development of rates of depression around adolescence. Although the origin of this steep rise in incident rates of depression and other disorders is unknown, several studies have suggested that exposure to stress during adolescence may be a relevant factor contributing to an individual's vulnerability to various mental disorders later in life (Friis et al., 2002; Ge et al., 2006; Bouma et al., 2008; McLaughlin and Hatzenbuehler, 2009).

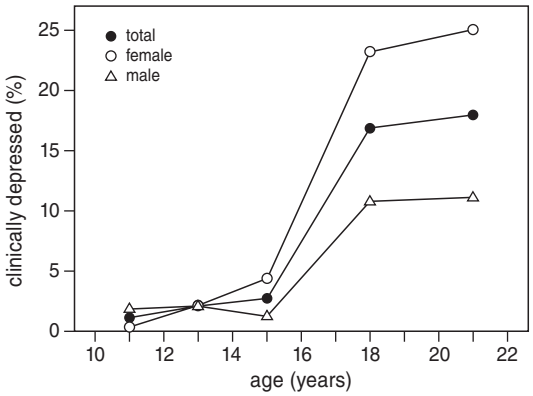

Figure 1.4 Development of overall rates of clinical depression by age and gender (adapted from Hankin et al., 1998).

In view of the dynamic changes in the prefrontal cortex during adolescence, it seems plausible that stress during this period may modulate prefrontal cortex functioning and can in this way have enduring behavioral consequences later in life. Indeed, exposure to uncontrollable stress during this developmental period is known to change prefrontal cortex functioning in humans (Arnsten, 2009).

Given the major social, psychological and neurobiological changes taking place during adolescence, this developmental period is of particular interest in this thesis.

INDIVIDUAL DIFFERENTIATION IN STRESS RESPONSIVENESS

The social environment is an important source of stress. In adolescent human beings this has often the form of bullying. There are large individual differences in the consequences of bullying in humans. Some persons report that they do not suffer from any negative consequences after experiencing bullying, whereas others report long lasting negative emotions (Ortega et al., 2009). This shows that some individuals are more vulnerable to bullying whereas others seem to be resilient. This is consistent with the well-recognized phenomenon that individual differences in sensitivity to stress are a critical factor in the development of psychopathologies both in humans and in animals (Schmidt et al., 2008; Kotov et al., 2010). However, the mechanisms underlying these individual differences in susceptibility to the consequences of bullying are poorly understood.

It is widely acknowledged in biomedical research that not the average population, but rather a small proportion of the human population is vulnerable to psychological disorders. For example, only 20-30% of individuals exposed to a serious traumatic event develop post-traumatic stress disorder (PTSD) (Breslau et al., 1991; Cohen et al., 2004).

There are also large individual differences in response to the same treatment; approximately fifty percent of all patients show full remission after treatment for depression (Nestler et al., 2002). The depressive symptoms disappear in some individuals whereas others do not respond or even get worse with treatment (Uher, 2011). For that reason, understanding individual disease vulnerability and personalized medicine has become a major area of research in the biomedical sciences (Ginsburg and Willard, 2009). Therefore individual differences in stress vulnerability and synaptic plasticity are studied in this thesis.

EXPERIMENTAL APPROACH

The hypothesis that adolescent social stress alters adult coping style and that this is mediated by BDNF-regulated synaptic plasticity in the prefrontal cortex was tested in an animal model. Social defeat was used as a model to study adolescent social stress. The consequences of social defeat on the adult capacity to deal with social challenges were determined in terms of offensive aggression, response to social defeat and impulsivity. Levels of offensive aggression were determined in a standard resident-intruder paradigm.

Furthermore, impulsivity was tested using several operant conditioning paradigms. In the next paragraphs I will describe in more detail the animal model and background of the behavioral experiments performed in this thesis.

ANIMAL MODELS

To study of the consequences of adolescent social stress on adult aggression and impulsivity at the level of synaptic plasticity animal models are required that allow precise control of the experimental variables. The resident-intruder paradigm in rodents is such a model. This paradigm is based on the fact that an adult male rat will establish a territory when given sufficient living space. As a consequence of territoriality, the resident will attack unfamiliar males intruding in its home cage. The intruder in turn will show defensive behavior in response to the offensive attack by the resident. Hence, offensive aggression and social stress can be studied using this paradigm (Koolhaas et al. in prep.).

In a normal population of rodents there are large individual differences in the level of (offensive) aggressive behavior. In the experiments described in this thesis we used animals of the Wild-type Groningen (WTG) strain (*Rattus Norvegicus*) which are characterized by large individual differences in aggressive behavior. WTG rats are descendant of four pairs of wild-trapped animals that are out-bred under conventional conditions in our laboratory. Within any batch of animals, individual male resident WTG rats differ widely in their level of species-typical offensive aggression expressed in their territory towards an unfamiliar intruder male. Some animals show no aggression at all, whereas others show high levels of aggressive behavior. Some of the animals show even pathological forms of aggressive behavior after repeated winning experience (Koolhaas et al., 1999; De Boer et al., 2003; Koolhaas et al., 2007; De Boer et al., 2009a).

Several studies in a number of animal species show that the individual variation in aggression is related to the way animals more generally cope with environmental stressors or coping style. Coping styles in animals are considered to be the equivalent of personality in humans and can be defined as a suit of correlated behaviors that are stable over time and across situations (Koolhaas et al., 1999). In general, proactive animals act based on predictions, are rigid, but act adequately in a stable environment and are fast but inaccurate. Whereas reactive coping animals first think, before acting, are flexible and adequate in a variable environment and are relatively slow, but accurate in their decisions.

Steimer has suggested a two-tier model of coping styles that consists of two independent axes (figure 1.5) (Steimer et al., 1997). This model allows the characterization of individuals on two independent scales in a two-dimensional space. The coping style axis reflects how an animal responds to a challenge (qualitative dimension) and an emotional reactivity axis reflects how strongly it responds (quantitative dimension). WTG rats have been shown to differ mainly in coping style. For the work described in this thesis, we used rats of the Roman selection lines as well. Rats of the Roman selection lines have been genetically selected for their performance in two-way avoidance (Bignami, 1965) and differ largely in their level of emotional reactivity (Steimer et al., 1997). Roman high

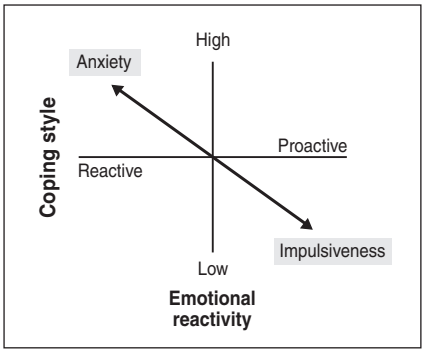

Figure 1.5 The two-tier model of coping styles. This model suggests that impulsiveness is associated with a proactive coping style and low emotional reactivity (X-axis). Anxiety on the other hand results from the combination of increased emotional reactivity with a passive coping style (adapted from Steimer et al., 1997).

avoidance (RHA) rats perform well in two-way avoidance and adopt a proactive coping style when confronted with a novel environment. They show little anxiety in novel situations and tend to be impulsive and novelty (sensation) seekers. Roman low avoidance (RLA) rats show a more reactive coping style during these tests (Fernandez-Teruel et al., 1997; Fernández-Teruel et al., 2002; Steimer and Driscoll, 2003).

TESTING IMPULSIVITY

Based on the data in human studies and the involvement of the prefrontal cortex in aggressive behavior (Blair, 2004; Siever, 2008) we hypothesize that aggressive animals are impulsive as well. Operant conditioning tasks are often employed to test impulsivity. In these tasks, animals are trained to perform an operant (usually to press a lever or turn a wheel) to trigger reinforcement (usually food or water). An operant conditioning paradigm allows precise experimental control of the relationship between stimuli and responses. Hence, a more refined analysis of the various behavioral control mechanisms that determine behavioral flexibility is possible. Lack of behavioral flexibility can be considered as an important component of impulsivity. Behavioral flexibility reflects the degree to which behavior is guided by stimuli from the environment, which can be considered as an important fundamental and rather stable differential characteristic of coping styles. The most common tasks to analyze differences in impulsive behavior are the delay discounting paradigm, differential-reinforcement-of-low-rate (DRL) responding procedure or an autoshaping procedure (see for review (Monterosso and Ainslie, 1999)). In the experiments described in this thesis we used in addition to the DRL procedure a variable interval schedule to test differences in behavioral flexibility.

SOCIAL DEFEAT MODEL

The social environment is an important source of stress (conflicts and tension) in the everyday life of humans. To induce social stress in rats, we used the social defeat paradigm. Björkvist (Bjorkqvist, 2001) suggested that the social defeat paradigm is an ecologically valid model to study the consequences of social stress, victimization and social subjugation. Social defeat has been shown to induce long-lasting behavioral, physiological, and neurobiological changes. These include changes in social anxiety, heart rate, body temperature, activity as well as structural and functional changes in various brain neurocircuitries of rats (Meerlo et al., 1999; Buwalda et al., 2005).

Social defeat in adolescence has been shown to induce long-term behavioral changes, such as increased anxiety (Tsoory et al., 2007; Vidal et al., 2007; Toth et al., 2008a; Vidal et al., 2011b) and an altered behavioral response to amphetamine in adulthood (Burke et al., 2010). Repeated social stress during adolescence in male golden hamsters has been shown to accelerate the onset of adult-like aggressive behavior and the level of attack frequency in early adulthood is higher in stressed animals (Delville et al., 2003; Wommack et al., 2003; Wommack and Delville, 2007).

I used the social defeat model to study the consequences of adolescent social stress on adult coping style and on synaptic plasticity in this thesis.

OUTLINE OF THIS THESIS

The main aim of the work described in this thesis was to study the consequences of adolescent social stress on adult aggression and impulsivity, with special emphasis on individual differences in susceptibility. Furthermore, I tested the hypothesis that the behavioral outcome of social defeat experienced during adolescence is modulated by synaptic plasticity in the prefrontal cortex.

The aim of **chapter 2** was to show that individual differentiation in behavior emerges as a function of underlying variability in the activation of brain circuitries supporting behavior. For example, individual differences in behavioral flexibility are hypothesized to be the result of differences in prefrontal cortex functioning and its key neurochemical signaling pathways (e.g. dopaminergic and serotonergic input).

I hypothesized that aggression and impulsivity are two related behavioral constructs. We studied the association between aggression, impulsivity and emotional reactivity in the experiments described in **chapter 3** and **4**. The standard resident-intruder paradigm was used to determine the level of offensive aggression, while several operant conditioning paradigms were employed for analysis of impulsive behavior.

The question whether individual differences in impulsivity are accompanied by differences in the cardiovascular response of animals was the topic of **chapter 5**. I analyzed the relationship between performance during several operant conditioning tasks and the physiological response. Both heart rate and blood pressure were determined in relation to lever press performance during both reinforced and extinguished operant conditioning sessions.

I expected that disturbance of normal prefrontal cortex development by adolescent social stress leads to long-term changes in adult coping style in WTG rats and rats of the Roman selection lines. In **chapter 6** and **7**, I studied the effects of adolescent social defeat and focused particularly on adult prefrontal cortex functioning, i.e. aggression and impulsivity.

The aim of **chapter 8** was to study whether adolescent social stress leads to structural and functional reorganization of the neural circuitry underlying stress reactivity. Furthermore, I aimed at comparing the effects of adolescent social stress on synaptic plasticity to the effects of social defeat stress in adulthood.

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CHAPTER 2

Coping styles and behavioral flexibility: towards underlying mechanisms

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ABSTRACT

A coping style (also termed behavioral syndrome or personality) is defined as a correlated set of individual behavioral and physiological characteristics that is consistent over time and across situations. This relatively stable trait and universal phenomenon characterizing a broad range of species confers differential adaptation to environmental conditions. Behavioral flexibility appears to be an important underlying attribute or feature of coping style that might explain consistency across situations. Proactive coping is characterized by low flexibility expressed as rather rigid, routine-like behavioral tendencies and reduced impulse control (behavioral inhibition) in operant conditioning paradigms. This manuscript summarizes some of the evidence that individual differentiation in behavioral flexibility emerges as a function of underlying variability in the activation of a brain circuitry that includes the prefrontal cortex, and its key neurochemical signaling pathways (e.g., dopaminergic and serotonergic input). We argue that the multidimensional nature of animal personality and the terminology used for the various dimensions should reflect the differential pattern of activation of the underlying neuronal network and the behavioral control function of its components. Accordingly, unravelling the molecular mechanisms that give rise to individual differences in coping style will be an important topic in biobehavioral neurosciences, ecology and evolutionary biology.

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INTRODUCTION

During the last decades, a wide variety of scientific disciplines has shifted its interest towards the causes and consequences of individual variation. Ecologists and evolutionary biologists aim at understanding the ecological function of individual variation in behavior and its consequences for evolutionary fitness (Sih et al., 2004a; Reale et al., 2007; Wolf et al., 2008). Understanding individual disease vulnerability and personalized medicine has become a major area of research in the biomedical sciences (Ginsburg and Willard, 2009), and in the behavioral neurosciences much research effort is devoted to gene-environment interaction in the development of adult phenotypes and the underlying physiological mechanisms (Barr et al., 2003). Although the boundaries between these disciplines gradually disappear, we feel that much can be gained by a further integration both at the conceptual level and the design of experiments.

Naturalistic studies in a variety of animal species show that individuals can be categorized in distinct behavioral phenotypes. These studies are all based on two observations; a) within an individual, behaviors are often correlated independent of the environmental situation, and b) correlated behaviors result in only a limited number of phenotypes across individuals. Several terms are used for this phenomenon. Sih et al. (2004) used the term behavioral syndrome (Sih et al. 2004), whereas Groothuis and Carere preferred the term behavioral profile (Groothuis and Carere, 2005).

More specifically, research has focused on two distinct patterns of reaction to stressful conditions or coping style. Rodent research distinguishes between proactive and reactive coping (Koolhaas et al., 1999) and researchers of fish and birds often use the terms shyness and boldness (Sloan Wilson et al., 1994). Whatever term is used exactly, they all refer to alternative response patterns in reaction to challenges that are stable over time and across various situations (Koolhaas et al., 1999). For example, animals characterized by a proactive coping style, are offensive towards male conspecific rivals, are impulsive in decision making, score high in frustration tests, take risks in the face of potential dangers and are novelty seekers (David et al., 2004; Groothuis and Carere, 2005; Steimer and Driscoll, 2005).

Although an uni-dimensional approach of individual variation is useful in these early stages of animal personality research, several studies emphasize the need to consider individual variation being composed of several independent trait characteristics (Steimer and Driscoll, 2005; Van Reenen et al., 2005; Koolhaas et al., 2007). These authors suggest a two-tier model in which a coping style axis reflects how an animal responds to a challenge (qualitative dimension) and emotional reactivity axis reflects how strongly it responds (quantitative dimension). This allows the characterization of individuals on two independent scales in a two dimensional space.

In view of the tests used to characterize individual fish or birds as shy or bold, it is conceivable that this phenotypic characterization includes both qualitative and quantitative aspects. The dimensions are generally determined using principle component analyses of the variation in behavior between individuals tested in various conditions. In human personality research this has resulted in five independent dimensions (the big five) or axes at

which individuals may vary (Goldberg, 1990). The fact that individual variation in behavior can be reduced to variation in a limited number of independent dimensions is important.

From an evolutionary perspective, variable trait characteristics are subject of selection pressure. Hence, the various dimensions may reflect independent components of individual fitness. From the point of view of behavioral neuroscience, it is reasonable to suggest that these dimensions somehow reflect underlying causal mechanisms. The idea is that certain behaviors are correlated because they share the same neurobiological, neuroendocrine and/or genetic mechanisms (Bell, 2007b; Bell et al., 2007).

The present paper aims at one of these causal mechanisms. Since we focus on individual behavioral characteristics that are stable across situations, one has to look for variation in causal mechanisms or behavioral control functions that are activated in different contexts in one and the same animal. Inter-individual variations in behavior in these contexts should consequently be reflected in a differential activation of the underlying causal mechanisms. This line of reasoning also implies that the dimensions used to describe individual variation in behavior should reflect variation in the main proximate mechanisms controlling behavior.

It is beyond the scope of this paper to review all causal mechanisms underlying individual trait characteristics. We will rather explore the neurobiology of behavioral flexibility as an important characteristic that might explain consistency of individual behavior across a wide variety of environmental conditions. We will mainly use data derived from rodent studies. An elaborate overview of the evolutionary basis of coping styles and the underlying physiology is given by Overli and co-authors (Overli et al., 2007). However, they do not specifically address the mechanisms of behavioral flexibility as defined below.

BEHAVIORAL FLEXIBILITY

Behavioral flexibility is an ill-defined concept. Evolutionary ecology uses the term behavioral plasticity to indicate that the expression of behavioral traits is not fixed within genotypes or individuals (Dingemanse et al., 2007). Applied to individuals, behavioral plasticity is defined as the slope of the relationship between behavior (response variable) over an environmental gradient: a behavioral reaction norm. This slope can thus be viewed as an index for the number of phenotypes a single genotype can produce in a given set of environments (Dingemanse et al., 2009).

Behavioral neuroscience does not use the concept of behavioral reaction norm. In this field of science, behavioral flexibility includes a range of behavioral control functions of an animal aimed to directly respond and adjust its behavior to environmental stimuli. This includes impulsivity (impulsive action/ impulsive choice), reversal learning/ response perseveration, etc. (Dalley et al., 2004).

Behavioral flexibility is defined as the ability of an individual to directly respond and adjust its behavior to environmental stimuli. Here we will consider the individual variation in the underlying behavioral control functions. Behavioral flexibility reflects the

degree in which behavior is guided by stimuli from the environment, which can be considered an important fundamental and rather stable differential characteristic of coping styles.

So far, flexibility of behavior in relation to coping style has mainly been tested in laboratory settings using rodents. A wide range of studies suggest that actions of the proactive coping style are principally based on predictions of the actual environment. This is in contrast to the reactive coping style in which there is a more direct stimulus-response relationship. For example, rats or mice can easily be trained to run a maze for a food reward. After reaching a stable task performance, the reaction to a small change in the maze is often studied. In one experiment on mice, a small piece of tape was put on the floor in one of the alleys of the maze, while in another experiment the maze was turned ninety degree with respect to the extra-maze cues. In both experiments, the proactive coping males paid little or no attention to the change; i.e. there was no increase in time to complete the task and no increase in the number of errors made in the maze. Reactive coping males on the other hand started exploring the maze again and hence took much more time to get to the goal box and make more errors in the task (Benus et al., 1990). This suggests that the reactive coping style may be much more guided by environmental stimuli, while the proactive coping style seems to rely on routines.

Similar results were obtained in a study of coping styles in pigs. Piglets that struggle a lot in the back-test (proactive, high resisters) are less successful in reversal learning of a T-maze task compared to animals that hardly show any resistance (reactive, low resisters) in the back-test. High resisting, proactive coping, pigs had more difficulties in inhibiting their previously reinforced response, which is consistent with the idea that these animals rely on previous experience and develop routines (Bolhuis et al., 2004).

Differences in behavioral flexibility can be demonstrated in several other situations where the animal has to switch suddenly from a familiar situation to a new one. For example, the two coping styles differ strongly in the response to a twelve-hour shift in light/dark cycle. Proactive coping male mice stay in their original day-night rhythm for a few days after which their rhythm gradually shifts to the new cycle. Reactive coping males on the other hand start to shift their rhythm immediately; they are twice as fast in adapting to the new light-dark cycle as the proactive coping males (Benus et al., 1988). This suggests that the rhythm of the reactive animals is more determined by the extrinsic light/dark cycle. Similar studies in non-mammalian species are hardly available. However, in their work on Great Tits as an avian model of coping styles, Verbeek et al. also concluded that the fast-exploring (i.e. proactive) birds seem to rely on routines (Verbeek et al., 1994).

Besides maze tests, operant conditioning tasks are often employed as well to test for behavioral flexibility. In these tasks, animals are trained to perform an operant (usually to press a lever or turn a wheel) to trigger a reinforcement (usually a food reward). An operant conditioning paradigm allows precise experimental control of the stimuli that the animals can respond to, and of the responses they make. Hence, a more refined analysis of the various behavioral control mechanisms that determine behavioral flexibility is thus possible. These include impulsive responding or behavioral inhibition, response perseveration and attention.

One of the studies aimed at documenting the relationship between coping styles and behavioral flexibility using an operant conditioning paradigm has been performed in hamsters (Cervantes and Delville, 2007). High aggressive hamsters perform impulsively compared to low-aggressive hamsters in a two lever delay-discounting paradigm. High aggressive hamsters were more likely to press a lever for an immediate but small reward, whereas low aggressive animals showed a preference for a delayed but larger reward (Cervantes and Delville, 2007). In a similar study by David and coworkers, hamsters were trained in operant conditioning chambers for immediate reinforcement and were later tested for their response to a delayed reward. They showed that all animals increase their frequency of lever pressing initially. However, low-aggressive animals were able to adapt to the delay and showed a decreased rate of lever pressing per reward within five days, reaching a significantly higher feeding efficiency than the high-aggressive males (David et al., 2004). Similar results were obtained in a genetic model of coping styles; the Roman High (RHA) and Roman Low (RLA) avoidance rats. These animals were genetically selected for their avoidance behavior in an active shock avoidance paradigm. Extensive studies show that the Roman Low avoidance animals are also less aggressive in a social interaction test and are more efficient in a delayed reinforcement task than Roman High Avoidance rats (Zeier et al., 1978). Apparently, non-aggressive males are better in inhibiting their actions when required.

These experiments all demonstrate different aspects of behavioral flexibility. It is important to note that these aspects of behavioral flexibility all seem to be correlated with the individual level of aggression, suggesting that behavioral flexibility can be considered as a rather fundamental and presumably stable component of the coping style dimension. It might also explain the consistency of individual behavior across different contexts. A reduced capacity of behavioral inhibition will not only affect the way an animal deals with its social environment but also how it deals with food shortage. The proactive animal acts primarily on the basis of previous experience (feed-forward control), which is a fast but may be inaccurate. The reactive coping animal tends to rely more on the detailed information available in the environment, which may take time to acquire but likely more accurate information on current environmental conditions. This fundamental difference in behavioral control may also relate to the adaptive nature of the two coping styles. A proactive coping animal may be adapted to stable environmental conditions, whereas the reactive coping style may do better under variable and unpredictable environmental conditions.

Although it is intriguing to notice that the above mentioned experimental paradigms developed in behavioral neuroscience are reminiscent of the paradigms used to measure optimal foraging in behavioral ecology (Shapiro et al., 2008), these predictions have hardly been tested under more natural conditions. In the Great Tit model, food availability in the field was found to be a major determinant in the differential survival of fast and slow exploring animals (Dingemanse et al., 2004). In a recent field experiment, van Overveld and Matthysen showed that fast exploring juvenile Tits more rapidly invaded new food resources than slow exploring birds after a sudden drop in food availability (Van Overveld and Matthysen, 2010), consistent with our thesis. Clearly, this topic needs a much more

elaborate experimental approach using carefully characterized animals that preferably vary along only one dimension of personality.

CAUSAL MECHANISMS

As argued above, the dimensions of personality are likely to reflect individual variation in the pattern of activity of underlying causal physiological mechanisms. We feel that the terminology used to describe the dimensions of animal personalities should somehow be consistent with the behavioral control function of the underlying brain structures. Moreover, a careful analysis of the key components of these proximate mechanisms is not only essential for an evidence-based candidate gene approach of animal personalities, it is also important in unraveling variable trait characteristics that might be subjected to selection pressure.

Many studies have considered neuroendocrine parameters as part of such a common causal mechanism for behavioral syndromes. For example, the shy individual is usually considered to be characterized by a high reactivity of the Hypothalamus Pituitary Adrenocortical (HPA) axis. However, in view of the multidimensional nature of behavioral syndromes as discussed above, one has to ask the question how neuroendocrine mechanisms relate to these dimensions. This has been discussed in a recent review for the HPA axis, the Sympathetic Adrenomedullary (SAM) system and the Hypothalamus Pituitary Gonadal (HPG) system (Koolhaas et al., 2010). There, it is argued that, with the exception of the HPG axis, it is unlikely that there is a direct causal relationship between these neuroendocrine systems and the coping style dimension. In other words neither corticosteroids nor plasma catecholamines determine the qualitative type of behavioral response to a challenge. It is more likely that the activity of these neuroendocrine systems reflects individual variation at the emotionality axis. However, it is important to notice that both the HPA axis and the SAM system have an important function in the metabolic support of behavior as well. Therefore, it cannot be excluded that the magnitude of these physiological responses may be a direct consequence of differences in physical activity. Consequently, one has to consider the possibility that the correlations between behavioral syndromes and neuroendocrine stress reactivity are secondary to the individual differences in behavioral activity (Koolhaas et al., 2010).

The brain circuitry that has been associated with various personality dimensions is depicted in figure 2.1. The amygdala, hypothalamus and the periaqueductal gray are mainly involved in the emotional reactivity of the organism. The neuronal network involved in behavioral flexibility, involves the prefrontal cortex, the nucleus accumbens and their dopaminergic and serotonergic input. It is beyond the scope of this paper to review the available literature on the function of this circuitry in behavior in detail. A more extensive review of the role of this circuitry in cue dependency of behavior, habit formation and behavioral flexibility is given by Everitt and Robbins (Everitt and Robbins, 2005). Here, we will focus on the question to what extent individual variation in behavior on the coping style axis is related to variation in (components of) this latter neuronal circuitry.

PREFRONTAL CORTEX AND BEHAVIORAL FLEXIBILITY

Several of the tasks used to measure behavioral flexibility in rodents are derived from tests of prefrontal cortex functioning. In general, the prefrontal cortex (PFC) has been associated with both aggressive behavior (Blair, 2004; Siever, 2008) and various aspects of behavioral flexibility such as impulsive action and impulsive choice (Dalley et al., 2008). Similarly in birds, the nidopallium, which is considered the avian homologue of the mammalian prefrontal cortex, has an important function in choice behavior and optimal foraging (Matsushima et al., 2008).

In mammals, the PFC can be divided into several sub-regions, each with a somewhat different function in the control of behavior. Its involvement in aggressive behavior seems to be secondary to its primary role in behavioral inhibition, decision making, working memory and planning of behavior (Dalley et al., 2004). Lesions of the orbital PFC in rats induced an increase in impulsive behavior as measured by a reduced performance in a delayed reinforcement task and a preference for smaller and more immediate reward (Mobini et al., 2002). In view of the current discussion on proximate mechanisms of coping styles, we now will consider the question to what extent individual variation in behavior is reflected in variation at the level of the prefrontal cortex.

The prefrontal cortex receives important input from the evolutionary ancient neurotransmitter system serotonin originating in the dorsal raphe nucleus (DRN, see figure 2.1A). Throughout the animal kingdom, serotonin is involved in the regulation of aggression (Kravitz, 2000; Kravitz and Huber, 2003; Miczek et al., 2007) and seems to have an evolutionary well conserved function in behavioral flexibility as well (Kravitz, 2000; Evers et al., 2007). The serotonergic input of the prefrontal cortex plays a causal role in the individual variation in both aggression and behavioral flexibility. Low levels of serotonin in the PFC have been associated with both aggression and impulsive behavior at the level of the PFC (Van Erp and Miczek, 2000; De Boer et al., 2003; Winstanley et al., 2006b; Caramaschi et al., 2007; Miczek et al., 2007). Several studies show that proactive and reactive coping rats and mice differ in the serotonergic input of the prefrontal cortex. Rats with extensive experience of aggressive behavior have lower levels of release of serotonin (5-HT) in the prefrontal cortex (PFC) (Van Erp and Miczek, 2000; De Boer et al., 2003; Ferrari et al., 2003; Caramaschi et al., 2007; Miczek et al., 2007). Similarly, aggressive mice strains have significantly lower levels of 5-HT and its metabolite 5-HIAA in the prefrontal cortex (Caramaschi et al., 2007).

A decrease in serotonergic function has also been implicated in impulsive action in various paradigms of impulsivity in both humans and rodents (Roberts et al., 1994; Fletcher, 1995; Harrison et al., 1997; Crean et al., 2002; Homberg et al., 2007). In the five-choice serial reaction time task (5-CSRTT), a task that has been developed to test for the inhibitory control of behavior, 5-HT depletion has been found to increase premature responding (Harrison et al., 1997). In addition, administration of the 5-HT releasing agent *d*-fenfluramine has been shown to decrease premature responding in the 5-CSRTT (Carli and Samanin, 1992). The role of serotonin in behavioral inhibition is confirmed by the behavioral disinhibition induced by 5-HT lesions of the raphe nuclei in rats using a selec-

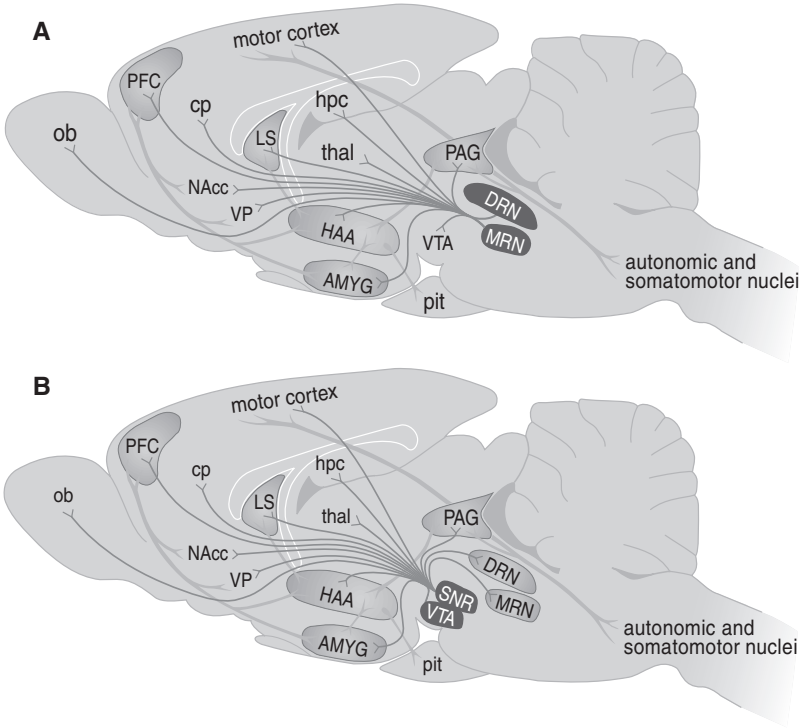

Figure 2.1 Overview of the main brain structures and their connections involved in (A) aggressive behavior and their serotonergic input from the dorsal raphe and (B) dopaminergic input from the ventral tegmental area. AMYG, amygdala; AVP, arginine vasopressin; cp, caudate putamen; DRN, dorsal raphe nucleus; HAA, hypothalamic attack area; hpc, hippocampus; LS, lateral septum; MRN, medial raphe nucleus; NAcc, nucleus accumbens; ob, olfactory bulb; PAG, periaqueductal grey; PFC, prefrontal cortex; pit, pituitary; SNR, substantia nigra; thal, thalamus; VP, ventral pallidum; VTA, ventral tegmental area.

tive neurotoxin (Fletcher, 1995). In the serotonin transporter (SERT) knockout rats, a continuously enhanced level of prefrontal cortex serotonin is associated with reduced aggression as measured in a resident-intruder paradigm. SERT knockout rats also show improved inhibitory control in a five-choice serial reaction task (5-CSRTT), but unchanged behavioral flexibility investigated in a reversal learning task (Homberg et al., 2007). Behavioral inhibition seems to be mediated by the medial PFC, because a delay discounting paradigm enhances 5-HT efflux in the medial PFC but not in the orbital PFC (Winstanley et al., 2006b).

Taken together, serotonin is causally involved in both aggression and behavioral flexibility. Individual variation in the serotonergic input to the medial PFC may explain the correlated individual variation in the coping style dimension. This is consistent with the hamster studies in an operant conditioning paradigm by Cervantes and Delville mentioned before in which aggressive hamsters had less 5-HT innervations of the PFC and were more impulsive than their non-aggressive counterparts (Cervantes and Delville, 2007; Cervantes and Delville, 2009).

MESOLIMBIC DOPAMINE SYSTEM AND REWARD PROCESSING

The fact that aggressive hamsters prefer an immediate small reward over a delayed large reward indicates that individuals may differ in processing of reward related cues (Cervantes and Delville, 2009). The mesolimbic dopamine system has an important role in processing of natural rewards. This system has its cell bodies in the ventral tegmental area (VTA) and innervates not only the nucleus accumbens (Nacc), but also the prefrontal cortex (figure 2.1B). This circuit is extensively studied for its involvement in natural reward processing and the development of drug addiction (Kelley and Berridge, 2002).
Several studies show that individual variation in coping with environmental challenges is related to differences at the level of this mesolimbic dopamine system. For example, in the Roman rat lines, the density of dopamine D1 receptors and D3 receptor binding in the NAcc is consistently higher in RHA than in RLA rats (Guitart-Masip et al., 2006; Giorgi et al., 2007). Furthermore, RHA rats show remarkable behavioral and neurochemical responses to the acute administration of morphine and psychostimulants (Corda et al., 2005; Giorgi et al., 2007) and are more susceptible, compared to RLA rats, to the reinforcing properties of cocaine (Fattore et al., 2009).

An extensive clinical and preclinical literature shows that impulsivity appears to be a major vulnerability factor in the development of substance abuse (De Wit, 2009). With regard to the argument of the present paper, these data support the view that individual differences in reward processing and the underlying neurobiology are important components of animal personality and behavioral flexibility that might explain the consistency of individual trait characteristics across contexts.

CONCLUDING REMARKS

The present paper argues that the behavioral expression of different coping styles, animal personalities or behavioral syndromes should be related to individual variation in the underlying causal neurobiological mechanisms. Behavioral flexibility seems to be an important component of individual differentiation in behavior that might explain consistency of individual differentiation across a wide variety of behaviors. Indeed, the lower flexibility observed in proactive coping animals as a reduced behavioral inhibition does explain not only short attack latencies in an aggressive interaction or an escape situation, but also the choice for immediate small rewards in a food related situation. Behavioral flexibility seems to relate to the degree in which behavior is guided by environmental input. The proactive individual behaves mainly on the basis of predictions, which is fast but can be inaccurate. At the same time, behavioral flexibility includes aspects of behavioral inhibition.

The medial prefrontal cortex has a key role in the neuronal network involved in behavioral flexibility and planning of behavior in time. An increasing number of studies show individual differentiation in the pattern of activation of the various components of this neuronal network in relation to phenotypic differences in behavioral flexibility. The func-

tional differentiation in dopaminergic and serotonergic input of the prefrontal cortex as discussed above is a prerequisite for a candidate gene approach of these two neurotransmitter systems. Indeed, several studies show that this might be a promising avenue. For example, polymorphisms in the promoter region of the serotonin transporter gene have been associated both with a functional change in the transporter capacity and with individual variation in aggression and personality in humans and in rhesus monkeys (Lesch and Merschdorf, 2000; Suomi, 2006). Similarly, a single nucleotide polymorphism in the gene coding for the dopamine 4 receptor has been associated with individual variation in novelty seeking and behavioral inhibition in humans and animals (Savitz and Ramesar, 2004; Munafo et al., 2008; Korsten et al., 2010). However, the nature of such a differentiation in neurobiology and underlying genetics in terms of independent dimensions of individual variation as discussed above has hardly been addressed. This would require an experimental approach of the question whether a manipulation in a certain component of the network affects behavioral characteristics of one dimension without affecting the characteristics of other dimensions. Such information is important to understand in more detail the individual behavioral characteristics that might be subjected to selection pressures.

Finally, it is tempting to consider the possibility that behavioral flexibility is a prerequisite for phenotypic plasticity at the within-individual level. Studies aimed at understanding individual stress vulnerability show that the behavioral flexible, reactive coping mouse shows the strongest stress-induced changes at the level of behavior, neuroendocrinology and neurobiology (Veenema et al., 2004). These changes have often been interpreted as signs of stress-induced pathology. However, these changes might just as well reflect the behavioral and physiological underpinning of individual adaptation. This line of reasoning suggests indeed that high behavioral flexibility is associated with a high capacity to adapt to a changing environment.

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CHAPTER 3

Aggression and aspects of impulsivity in wild-type rats

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ABSTRACT

Aggression is closely related to impulsive behavior both in humans and in animals. To avoid potential negative consequences, aggressive behavior is kept in control by strong inhibitory mechanisms. Failure of these inhibitory mechanisms results in violent behavior.

In the present experiments, we investigated whether aggressive behavior is related to impulsive behavior. Furthermore, we investigated if violent behavior can be distinguished from 'normal' aggressive behavior in terms of impulsivity levels.

We used rats of the wild-type Groningen strain, rats of this strain differ widely in their level of offensive aggression expressed towards an unfamiliar intruder male, ranging from no aggression at all to very high levels of intense and sometimes violent behavior. Violent behavior was displayed by part of the animals that were given repeated winning experience.

We used behavioral performance in an unpredictable operant conditioning paradigm for food reinforcement (variable interval 15) and performance in a differential-reinforcement of low rate (DRL-60s) responding as determinants for impulsivity.

We hypothesized that offensive aggression is correlated with behavioral flexibility measured with the VI-15 procedure and that aggressive behavior is characterized by low behavioral inhibition on the DRL task. In addition we expected that violent animals are characterized by low levels of behavioral inhibition on the DRL task.

We showed that the level of offensive aggression indeed positively correlated with VI-15 performance. In addition, we showed that behavior performance on the DRL procedure is similar in low and high aggressive. However, violent animals can be dissociated by a lower efficiency of lever pressing on a DRL-60s schedule of reinforcement.

INTRODUCTION

A major concern in our society is the prevalence of violence which stems from various forms of premeditated or impulsive forms of human aggression (Krug et al., 2002). In particular, recurrent outbursts of impulsive and unprovoked aggressive acts and displays are the cardinal symptoms of various well defined psychiatric disorders (Moeller et al., 2001) such as intermittent explosive disorder (Coccaro, 2000), borderline personality disorder (Goodman and New, 2000; Lieb et al., 2004), obsessive compulsive disorder (Chamberlain et al., 2005), addiction (Perry and Carroll, 2008), attention-deficit hyperactivity disorder (ADHD) (Chamberlain and Sahakian, 2007) and mania (Swann, 2009). Effective treatment of these disorders relies on a better understanding of the neurobiology of impulsive aggression, as it is generally acknowledged that functional or structural abnormalities in the neural circuitry implicated in aggressive behavior can increase the susceptibility for impulsive aggression and violence (Davidson et al., 2000; Coccaro et al., 2007; Nelson and Trainor, 2007; Siever, 2008).

From a biological point of view, aggression is a highly functional form of social behavior. It is involved in competition for resources and protection of the individual itself or offspring from conspecifics and predators (Ferrari et al., 2005). To avoid potential negative consequences, aggressive behavior is kept in control by strong inhibitory mechanisms (De Boer et al., 2003). Failure of these inhibitory mechanisms likely results in expressions of escalated aggression out of proportion to the provocation or context and consequently loses its adaptive function in social communication (De Boer et al., 2009a). In general, a lack of inhibitory control over behavioral acts is often considered to be an important trait-like characteristic of an impulsive-aggressive personality in humans (Coccaro, 1992; Manuck et al., 1998; Dolan et al., 2001).

Over the past decade, an increasing number of studies considered the biological basis of personality. Animal personality research defines personality as a suit of behavioral traits that are consistent over time and across situations (Sih et al., 2004b). In fact, each trait is considered as a dimension along which individuals may differ. In psychology, five broad dimensions (the 'big five') are used to describe human personality (Goldberg, 1990). It is likely that trait characteristics in animals are multidimensional as well.

In rodents, the individual variation in behavior has so far been summarized in a two tier model. One dimension of this model is a coping style dimension that reflects the way in which individuals more generally cope with challenging conditions. Offensive aggressive behavior, as a way to cope with a social challenge, loads on this coping style dimension (Koolhaas et al., 2010). Aggression is closely related to impulsive behavior both in humans and in animals (Cervantes and Delville, 2007; Rudebeck et al., 2007; García-Forero et al., 2009). However, it is unclear whether both aggression and impulsive behavior are components of the same trait and share a common axis of behavioral variability.

The prefrontal cortex (PFC) is a key node of the neurobiological circuitry implicated in emotional regulation and aggressive behavior (Davidson et al., 2000). The PFC is significantly involved in modulation of social behavior (Blair, 2004) and in control of mood (Drevets, 2000) and motivational drive (Ballard et al., 2011), functions that are important

components of the personality of an individual. During aggressive behavior the prefrontal cortex is activated as demonstrated by an activation of the immediate early gene cFos during and right after an agonistic encounter (Halasz et al., 2006; Haller et al., 2006). A reduced level of serotonin (5-HT) in the prefrontal cortex is associated with enhanced aggression, which is consistent with the serotonin (5-HT) deficiency hypothesis of escalated aggression (Van Erp and Miczek, 2000; De Boer et al., 2003; Ferrari et al., 2003; Caramaschi et al., 2007; Miczek et al., 2007; De Boer et al., 2009a). Similar functions of the prefrontal cortex and serotonin have been found in humans, for example the orbitofrontal cortex is involved in modulating reactive aggression (Cleare and Bond, 1995; Bjork et al., 2000; Blair, 2004). Therefore, from a neurobiological point of view, one may predict that individuals displaying high levels of aggression in a social context are more generally characterized by an inability to inhibit behavioral actions or responding in non-social situations.

Studies in rats and mice indicate that a lack of inhibition plays a central role in the transition of aggressive behavior into violence. Repeated winning of a fight induces violent behavior in a sub-population of aggressive rats (the 'winner effect'). These animals develop routines during the repeated fights and seem to act on predictions. With repeated experience, their behavior is hardly or not at all controlled by detailed environmental input. Consequently, they do not respond to submission signals and they will attack females and even anesthetized opponents. Low-aggressive animals act based on cues available in their environment and adapt their behavior to the situation (De Boer et al., 2009b). The association between individual differences in aggression and routine versus cue-dependent acting can also be demonstrated in non-social conditions, such as maze tasks. During maze experiments, aggressive male mice and rats do not, or hardly, react to changes in the environment and appear to form a rather rigid, routine-like behavioral pattern. Non-aggressive animals are more flexible and the behavior seems to be directed by details of the environment. For example, they react to both extra- as well as intra-maze changes (Benus et al., 1987; Benus et al., 1990; Benus et al., 1991). Besides maze tasks, operant conditioning tasks are frequently used to test individual differences in aspects of impulsivity. Several of these tasks are specifically developed to test prefrontal cortex function. Operant conditioning paradigms allow a precise experimental control of the stimuli an animal has to respond to.

Although both aggression and behavioral (dis)inhibition are believed to be dependent on prefrontal cortical functioning, it is unclear whether an aggressive personality is linked to behavioral disinhibition and impulsivity. Therefore, in the current experiment we explored the association between aggressive behavior and various aspects of impulsivity in rats, using an operant conditioning paradigm.

We used rats of the wild-type Groningen (WTG) strain, this strain is characterized by a wide naturally existing variation in coping style, i.e. response to social and non-social challenges. WTG rats differ widely in their level of offensive aggression expressed towards an unfamiliar intruder male, ranging from no aggression at all to very high levels of intense and sometimes violent aggressive behavior (De Boer et al., 2003). We hypothesized that this wide variation in offensive aggression in the WTG rats, is positively correlated with measures of impulsivity as assessed in reaction to a variable interval schedule of reinforce-

ment, extinction (frustrative non-reward) and a differential-reinforcement of low rate (DRL) responding procedure (for review (Monterosso and Ainslie, 1999)). In addition we expected that violent animals are characterized by extreme rigid behavior on the VI-15 schedule and extremely low levels of behavioral inhibition on the DRL task.

MATERIALS AND METHODS

Animals

Adult male wild-type Groningen (WTG) rats (*Rattus Norvegicus*; originally wild-trapped animals and bred under laboratory conditions for over 45 generations in our own facilities) weighing 458 ±12 gram were used. The level of offensive aggression differs widely among individuals of this strain, ranging from no aggression at all to almost pathological levels of aggressive behavior. They were housed in groups of 5-6 animals from weaning until the start of the experiments.

Animals were housed in temperature-controlled rooms (21 ±2°C) under a 12h reversed light:dark cycle (lights off at 12 am) with water available *ad libitum*. During operant conditioning, animals were food restricted to 85–90% of their free-feeding bodyweight. All experiments were approved by the Groningen University Committee on Animal Experiments.

Resident-intruder test

Wild-type Groningen rats were characterized for their level of offensive behavior using resident-intruder tests. Animals were housed in large observation cages (80×55×50 cm) with an oviduct-ligated female for one week to avoid social isolation and facilitate territorial behavior. After one week, the baseline level of aggressive behavior was tested in the resident-intruder test. One hour prior to testing, females were removed. During the first three tests an unfamiliar Wistar male (intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was measured. The intruder was removed after the first attack. When no attack occurred within 10 minutes, the intruder was removed. During the fourth test, the full range of behaviors was scored during 10 minutes after the first attack. The frequency and duration of behavioral elements were scored. A total of 12 behavioral acts and postures were scored and grouped in 5 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration* (moving towards, nosing, investigating opponent, anogenital sniffing, crawl over, attempted mount, social groom); 3) *Non-social exploration* (ambulation, rearing, sniffing, scanning, digging); 4) *Inactivity* (sitting, lying, immobile, freezing); 5) *Grooming* (washing, shaking, scratching). The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals. For correlations with operant conditioning, we used the time spent on offensive behavior.

Repeated winning experience

For the DRL-60 experiment a selection of low aggressive and medium/high aggressive ani-

mals were exposed to eight additional resident-intruder sessions. During the last session the full behavioral profile was scored as described above. In addition, the attack latency to an intruder in an unfamiliar environment was scored and the latency to attack a female. Both measures are considered to test pathological aggression.

Operant conditioning apparatus
Operant conditioning equipment (Med Associates Inc., St. Albans, VT) was installed in the home cage of the animals. Sawdust was used as bedding material. One retractable lever was located next to a food receptacle. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms.

Operant conditioning
The operant conditioning procedure was divided in different phases: acquisition on a fixed ratio schedule, training on a variable interval scheme and responding during extinction. Sessions were conducted daily. Animals were maintained at 85–90% of their original body weight using an additional amount of chow supplied a few hours after the daily operant conditioning session.

Training
Animals were singly housed in operant conditioning cages, normal lab chow was removed and the retractable the lever was extended into the chamber and delivered pellets under a fixed ratio 1 schedule (FR-1). On the first day of training 20 pellets were distributed into the food receptacle and 5 pellets were placed on the lever to facilitate lever pressing. After 24 hr on a FR-1 schedule, the availability of the lever was reduced to one hour per day at the start of the dark phase for the total duration of the experiment. After one week, animals received seven sessions of reinforcements according to a variable interval 15 (range 2-32 sec) schedule (VI-15) for 7 days.

Extinction
One extinction session was performed after the variable interval training. During extinction, the lever was extended and responding still resulted in activation of the food dispenser, but the food dispenser was disconnected and no pellets were distributed. In this way all secondary stimuli were maintained.

Differential-reinforcement-of-low-rates (DRL)
A selection of low aggressive (<5% offensive aggression), high aggressive (>55% offensive aggression) and 'violent' animals (>55% offensive aggression, attacking a female and/or attacking in an unfamiliar environment) was made before the start of the operant conditioning sessions. Animals were first trained on a FR-1 schedule of reinforcement until all animals had obtained at least 100 rewards within one hour (after two days).

Subsequently, animals were trained in a DRL procedure during the first two hours of the dark phase. Initially, animals were trained to respond under a DRL 30-s schedule, which means that rats had to wait at least 30 seconds between two lever presses to obtain a food pellet. After stable performance, the schedule was increased to 60 seconds. Animals remained on a DRL 60-s schedule until stable performance (15 sessions). The total number of responses, total number of pellets obtained, efficiency (rewards/responses × 100) and food receptacle visits were analysed.

The inter-response time (IRT) of the last DRL 60-s session was analysed using peak deviation analysis (for procedure see Richards et al 1993). Briefly, the IRT distribution often shows a bimodal distribution with a peak at short IRT intervals and a second peak around the DRL value (in this case 60 sec). Peak deviation analysis divided the obtained IRT distribution into burst (IRT <6 sec) and pause (IRT >6 sec) components. The number of responses was compared to a theoretical distribution which predicts the appearance of the obtained IRT distributions if responses were emitted at the same overall rate, but randomly in time with respect to the preceding response. This expected curve is called the corresponding negative exponential. The corresponding negative exponential was computed based on the mean IRT of the obtained pause IRT durations excluding those obtained during the burst component (IRT <6 sec). This analysis resulted in three parameters: the peak area (PkA), peak location (PkL) and the burst ratio (BR). The burst ratio was used to characterize the tendency of the animal to respond in bursts. The PkA and PkL were used to characterize the pause distribution. The peak is the area of the obtained IRT distribution above the corresponding negative exponential. The largest possible PkA value (1.0) only occurs if all the obtained IRT intervals have exactly the same value, whereas the smallest PkA value (0) indicates that the obtained IRT distributions and corresponding negative exponential are identical. Thus, decreases in PkA indicated that the IRT distribution becomes more similar to random performance, indicating loss of schedule control. The peak location is the median of the area of the obtained IRT distribution above the corresponding negative exponential.

Statistics

To investigate the relationship between offensive behavior and operant conditioning behavior was analysed by calculating Pearson`s correlation coefficients. Results of selected low aggressive, high aggressive and violent WTG rats were compared using ANOVA's. A Tukey test was done as *post hoc* analysis. A *p*-value smaller than 0.05 was considered to be statistically significant.

RESULTS

Fixed ratio, variable interval training and extinction

Performance under the FR-1, VI-15 schedule of reinforcement and extinction is summarized in table 3.1. No significant individual differences were found during a fixed ratio schedule of reinforcement. Hence, the number of lever presses and obtained rewards did

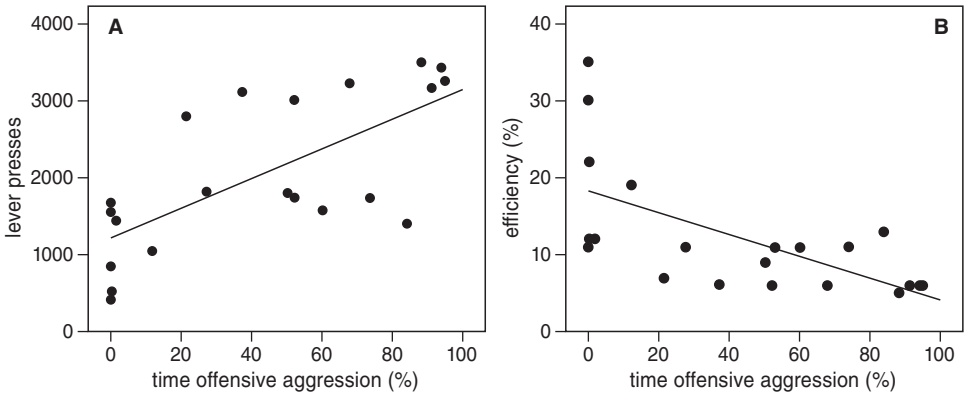

Figure 3.1 (A) Time spent on offensive behavior in WTG rats as measured in the resident intruder paradigm and the number of lever presses on a VI-15 schedule of reinforcement (correlation: R = 0.70, n = 21, $p < 0.001$). Percent of variability explained by the regression is found by squaring the R value. (B) Time spent on offensive behavior and the efficiency of lever pressing on a VI-15 schedule (correlation: R = -0.63, n = 21, $p < 0.01$).

not correlate with aggressive behavior ($p = 0.38$), neither did the number of food receptacle visits during this reinforcement schedule ($p = 0.86$).

On a variable interval schedule of reinforcement, individual performance differed widely and the number of lever presses was positively correlated with the percentage time spent on offensive aggression during the resident-intruder test (r = 0.70, $p < 0.001$). The efficiency of lever pressing (number of pellets/number of responses multiplied by 100) was negatively correlated with offensive behavior ($p = 0.002$) (figure 3.1). The number of obtained rewards and the frequency of food receptacle visits was not correlated with offensive behavior (r = 0.31, $p = 0.17$ and r = 0.33, $p = 0.15$ respectively).

No significant correlations were found in responding and food receptacle visits during extinction. However, a trend towards a positive correlation between offensive aggression and lever pressing was apparent during extinction (r = 0.41, $p = 0.06$).

Table 3.1 Lever press behavior during the FR-1, VI-15 and extinction of lever pressing paradigms. Pearson correlation coefficients (r_p) between aggressiveness (% time offensive behavior) and lever press parameters (n = 21).

	FR-1		VI-15		Extinction	
	Mean ± SEM	r_p	Mean ± SEM	r_p	Mean ± SEM	r_p
Lever presses	190 ± 9	0.20	2056 ± 218	0.70***	689 ± 58	0.41
Rewards	190 ± 9	0.20	184 ± 3	0.31	--	--
Food tray visits	287 ± 24	-0.04	526 ± 61	0.33	190 ± 31	0.20

*** Indicates a significant difference ($p < 0.001$)

Differential-reinforcement-of-low-rate responding

The levels of offensive aggression and the measures for pathological aggression are shown in table 3.2. The attack latency was significantly different but not between high aggressive and violent animals ($F_{2,17} = 19.46$, $p < 0.001$). The level of offensive aggression was significantly different as well, in a similar way ($F_{2,17} = 54.45$, $p < 0.001$). The latency to attack a female ($F_{2,17} = 12.70$, $p < 0.001$) and in an unfamiliar environment ($F_{2,17} = 53.28$, $p < 0.001$) was lower in violent animals.

Stable performance on a DRL 60-s schedule of reinforcement was acquired after 15 sessions of daily training. Analysis was done on the last day of DRL 60-s training. Behavioral performance on the DRL 60-s schedule is summarized in table 3.3. A trend towards a significantly different number of lever presses was found ($F_{2,17} = 3.43$, $p = 0.06$). The number of obtained rewards ($F_{2,17} = 1.97$, $p = 0.17$) and food receptacle visits ($F_{2,17} = 0.52$, $p = 0.60$) did not differ between the groups. The number of lever presses per reward was significantly higher in violent animals compared to low aggressive animals ($F_{2,17} = 3.76$, p=0.05) (figure 3.2).

The burst ratio ($F_{2,17} = 0.44$, $p = 0.65$), peak area ($F_{2,17} = 0.12$, $p = 0.89$) and peak location ($F_{2,17} = 0.52$, $p = 0.60$) did not differ between groups. The IRT distribution and peak deviation analysis of low aggressive, high aggressive and violent animals is shown in figure 3.3.

Table 3.2 Aggression data of the three different groups ($n = 6$ per group) of WTG rats used for the study. Data are expressed as mean ± SEM. Attack latencies are in seconds, offensive aggression is expressed as percentage time of a ten minute test.

	Low aggressive	High aggressive	Violent
Attack latency	418 ± 71	64 ± 13[a]	29 ± 11[a]
Offensive aggression	12 ± 5	59 ± 3[a]	72 ± 2[a]
Attack latency female	600 ± 0	553 ± 40	198 ± 83[a,b]
Attack latency unfamiliar environment	600 ± 0	557 ± 31	151 ± 40[a,c]

[a] Indicates a significant difference with low aggressive animals ($p < 0.001$)
[b] Indicates a significant difference with high aggressive animals ($p < 0.01$)
[c] Indicates a significant difference with high aggressive animals ($p < 0.001$)

Table 3.3 Lever press parameters during stable DRL 60-s performance. Data are expressed as mean ± SEM ($n = 8$ per group).

	Low aggressive	High aggressive	Violent
Lever presses	138 ± 9	154 ± 15	191 ± 18
Rewards	45 ± 8	37 ± 3	30 ± 2
Food tray visits	202 ± 39	216 ± 37	167 ± 30

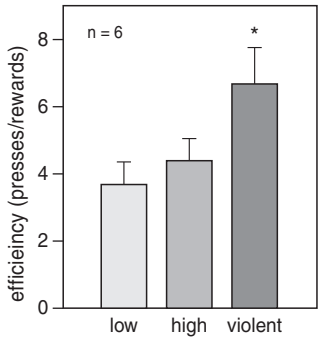

Figure 3.2 Lever pressing efficiency expressed as lever press responses per reward earned during the last DRL-60s session. Data are expressed as mean ± SEM. *$p < 0.05$.

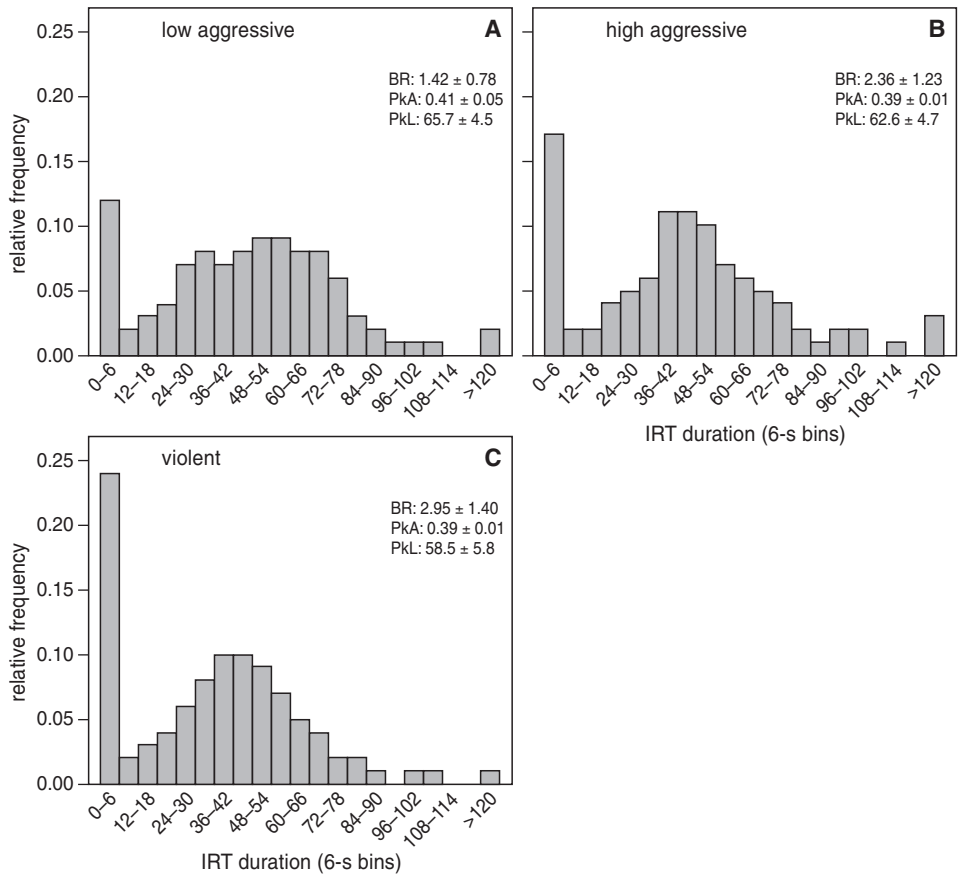

Figure 3.3 Inter-response time distributions and peak deviation analysis of the last DRL 60-s schedule of reinforcement in WTG rats. (A) Low aggressive animals ($n = 6$). (B) High aggressive animals ($n = 6$). (C) Violent animals ($n = 6$).

DISCUSSION

The present results show that aggression is associated with impulsivity as measured with a variable interval schedule of reinforcement during operant conditioning. This is not accompanied by a correlation in the number of rewards obtained or the number of food receptacle visits in the WTG rat. In terms of a cost/benefit analysis of operant conditioning behavior, high aggressive animals are less efficient (Hursh et al., 1988). This shows that high aggressive animals are less adapted to a variable and unpredictable environment and low aggressive animals are doing better under these conditions.

During extinction (frustrating non-reward), all animals reduced the number of lever presses and individual differences in the number of responses were less abundant. However, there is a trend towards a correlation between the number of lever presses and the time spent on offensive behavior.

Results obtained by using the WTG rat are in accordance with studies performed in Wistar rats (Van den Bergh et al., 2006) and hamsters. High aggressive hamsters show more impulsive behavior in the delay discounting paradigm compared to low aggressive hamsters (David et al., 2004; Cervantes and Delville, 2007). Comparable results in terms of behavioral inhibition have been obtained in studies using maze experiments in mice and pigs. Mice selected and bred for short attack latencies show routine-like, inflexible behavior in maze tasks (Benus et al., 1987; Benus et al., 1990; Benus et al., 1991).

Similar results were obtained in a study of coping styles in pigs. Piglets that struggle a lot in the back-test (proactive, high resisters) are less successful in reversal learning of a T-maze task compared to animals that hardly show any resistance (reactive, low resisters) in the back-test. High resisting, proactive coping, pigs had more difficulties in inhibiting their previously reinforced response, which indicates the development of routine-like behavior (Bolhuis et al., 2004).

Measures of performance under a DRL schedule of reinforcement are assumed to reflect various aspects of impulsive behavior. Bursting on a DRL schedule may be due to a lack of feedback for unreinforced responses and can be seen as behavioral disinhibition. The peak area and peak location indicate the behavioral control over the imposed DRL scheme (Richards et al., 1993).

Low aggressive animals and high aggressive animals were equally efficient on the DRL-60 schedule of reinforcement. Both in humans and in animals different measures of impulsivity are not correlated within individuals, for example tasks involving delays and response inhibition are shown to be independent from each other (McDonald et al., 2003; Winstanley et al., 2004b; Van den Bergh et al., 2006).

Violent animals were less efficient on the DRL-60 schedule of reinforcement. This indicates that violent animals may be distinguished from other animals by a disturbed ability to wait for a reward. This might show that a lack of impulse control may be an important aspect of the pathology of aggression.

The response rate, number of obtained rewards, food visits and the shape of the inter-response time (IRT) distribution, that is characteristic for DRL responding were compared. None of these parameters were different between low aggressive, high aggressive and

violent animals.

A comparable DRL paradigm has been performed with rats of the Roman selection lines. Rats of these artificial genetic selection lines show two strikingly distinct coping styles. Proactive coping Roman High Avoidance (RHA) rats have been shown to be sensation seekers and behave impulsively (Steimer and Driscoll, 2003; Moreno et al., 2010). Under a DRL 20-s schedule of reinforcement, RHA rats are less capable of inhibiting non-relevant activity, although this effect was mainly seen in female rats and not in male rats (Zeier et al., 1978).

Impulsivity is a broad defined concept and consists of several independent dimensions including decreased inhibitory control, intolerance to delay to rewards and quick decision making due to a lack of evaluation of the environment (Evenden, 1999). The current experiments show that the individual level of offensive aggression is associated with some of these aspects of impulsivity (i.e. performance on a variable interval schedule and extinction and not with others (tolerance to delay of rewards and bursting). Furthermore, violent animals may be distinguished from normal aggressive animals by a disturbed ability to wait for a reward.

We hypothesize that the association between aggression and impulsivity might be explained by the involvement of a common neuronal substrate such as the prefrontal cortex. Whether a causal relationship exists between aggression and impulsivity and if this is solely dependent on prefrontal cortex functioning needs further investigation.

Our results are consistent with the idea of Pattij and colleagues that different behavioral phenomena of impulsivity might rely on separate neural pathways (Pattij and Vanderschuren, 2008). For example, lesions of the anterior cingulate cortex affect how much effort rats decided to invest for rewards, whereas orbitofrontal cortical lesions affect how long rats decided to wait for rewards (Rudebeck et al., 2006). These behavioral phenomena can be dissociated both neuro-anatomically as well as pharmacologically (Pattij and Vanderschuren, 2008).

Furthermore, similar behavioral performance during operant conditioning tasks in animals with different coping styles might be explained by collateral behaviors (Rawlins et al., 1983); animals might use different strategies to come to the same behavioral output. Whereas some animals may depend on the prefrontal cortex to perform well on a DRL schedule of reinforcement, other animals may rely more on the hippocampus or striatum and come to the same behavioral output (Cho and Jeantet, 2010).

Remarks

One may argue that differences in responding are caused by differences in activity and not by differences in impulsivity. However, during the training phase in which a predictable schedule of reinforcement was used (fixed ratio schedule) offensive aggression was not correlated with lever pressing. In contrast to a delay discounting paradigm, no learning is involved on the variable interval schedule of reinforcement, since this schedule of reinforcement is completely unpredictable. During training on the DRL schedule of reinforcement we increased the delays at once to 30 seconds and thereafter to 60 seconds instead of increasing the delays progressively. Pizzo and colleagues have shown that by increment-

ing the DRL criterion over sessions may be an inefficient way of training stable DRL performance (Pizzo et al., 2009).

We would like to add some remarks with respect to the experimental procedures. Firstly, in contrast to most research done using operant conditioning, we performed our experiments in the home cage of animals. This reduces stress related with transfer to the operant conditioning cage and allowed us to test animals in their common laboratory environment. Secondly, food restriction seems to be of major importance for the motivational effects of the studies. Under conditions in which food is not restricted animals do not show a difference in responding during operant conditioning tasks (unpublished observations). This means that only under certain circumstances differences in impulsivity are expressed.

Functional significance

Impulsivity can both be both beneficial and detrimental. In case rapid decisions have to be made, it might be good to show some form of impulsive behavior, whereas recurrent high levels of impulsivity are associated with psychiatric disorders such as ADHD, substance abuse and personality disorders (American Psychiatric Association, 1994).

Similar characteristics (inability to reduce responding in an unpredictable environment, but normal performance during a delay task) that we found in our normal aggressive WTG rats are seen in antisocial personality disorder. This is a common and potentially dangerous disorder, characterized by poor impulse control and destructive behavior that begins in childhood and persists into adulthood (American Psychiatric Association, 1994).

Antisocial personality disorder is characterized by increased rapid-response impulsivity (inability to evaluate a stimulus before responding to it), whereas the level of reward-delay impulsivity (inability to delay responding despite a larger reward) is comparable to healthy subjects (Swann et al., 2009). Rats of the WTG strain might therefore be a valuable animal model of antisocial personality disorder, the cause and treatment of the disorder might be unravelled by using such animal models.

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CHAPTER 4

Correlated behavioral traits in rats of the Roman selection lines

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ABSTRACT

The current theories of animal personality are based on the observation that individual variation in behavior and physiology appears to be consistent across contexts. Rats of the Roman selection lines have been originally selected for differences in shuttle-box behavior. Besides differences in active avoidance, these animals differ more generally in coping style. Roman High Avoidance (RHA) rats show high levels of active avoidance, whereas Roman Low Avoidance (RLA) rats tend to respond with a more passive (i.e. freezing) response. Based on the two-tier model of coping styles, we hypothesized that RHA rats would show high levels of offensive behavior and are more impulsive compared to RLA rats.

We characterized animals in a two-way active avoidance task on five consecutive days. Thereafter animals were tested for their level of offensive aggression and impulsive behavior.

The level of offensive aggression was examined in a standard resident-intruder paradigm. Furthermore, we tested aspects of impulsivity in an unpredictable operant conditioning paradigm (variable interval-15 schedule) for food reinforcement and during extinction of lever press behavior.

We show that RHA rats are indeed characterized by high levels of two-way active avoidance in a shuttle-box paradigm. Surprisingly, the level of offensive aggression was higher in RLA compared to RHA rats. Consistent with the coping style interpretation, the number of lever presses in the VI-15 schedule for food reinforcement was higher in RHA rats compared to RLA rats. During a session of frustrating non-reward, RHA rats were more persistent.

Taken together, results of the two-way active avoidance task and VI-15 performance in rats of the Roman selection lines fit with the two tier model of coping styles. Unexpectedly, the level of offensive aggression does not match with this model.

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INTRODUCTION

The current theories of animal personality are based on the observation that individual variation in behavior and physiology appears to be consistent across contexts. For example, in a shock-prod defensive burying paradigm, which is extensively used as a test for fear/anxiety in preclinical studies, animals can either respond actively to the electrified shock prod that is inserted into their home cage by burying the prod or they can respond more passively by neglecting the prod or by freezing (De Boer and Koolhaas, 2003). Burying behavior in this test is positively correlated with offensive aggression measured in a resident intruder paradigm in wild-type Groningen rats (De Boer et al., 2003; Koolhaas et al., 2007). These correlated behavioral responses have been interpreted as coping styles, i.e. two qualitatively different response patterns to an emotionally arousing stimulus (Koolhaas et al., 1999).

Rats of the Roman selection lines are used as a genetic model of two strikingly distinct coping styles. These strains have originally been selected for high and low rates of two-way active shock avoidance conditioning (Bignami, 1965). The Roman high avoidance (RHA) rat is characterized by rapid acquisition of an active avoidance response, whereas the Roman low avoidance (RLA) rat acquires the conditioning slowly or even fails to show avoidance behavior. Consistent with the coping style interpretation, in the defensive burying test, RLA rats respond with immobility (Roozendaal et al., 1993), whereas RHA rats respond with burying the electrified shock prod (Boersma et al., 2009).

Apart from differences in shuttle-box behavior, these two lines of rats differ in coping with other behavioral challenges as well. When RLA rats are exposed to novel environments, they defecate more, show freezing behavior and display more self-grooming compared to RHA rats (Steimer et al., 1997; Escorihuela et al., 1999; Steimer and Driscoll, 2005). In anxiety related tests, such as the dark/light open field test, RLA rats are more anxious and the latency to cross the dark/light border is longer compared to RHA rats (Steimer and Driscoll, 2003).

In a summary of all behavioral results, Steimer and Driscoll (1997) have positioned the Roman selection lines in a two tier model with coping style and emotionality as the two dimensions. The RLA rat is characterized by high emotionality associated with a reactive coping style. On the other hand, the RHA rat is characterized by low emotional reactivity and a proactive coping style (Steimer et al., 1997; Steimer and Driscoll, 2003).

Proactive coping in combination with low emotional reactivity has been associated with impulsivity. Indeed, RHA rats have been shown to behave impulsively in several tests. In a five-choice serial reaction time (5-CSRT) task, RHA rats show more premature responses and fewer omissions compared to RLA rats (Moreno et al., 2010). Similar differences are shown in operant conditioning tasks. In a delay discounting paradigm, RLA rats chose the large, but delayed reward more often compared to RHA rats (Moreno et al., 2010). Likewise, during the acquisition of a differential reinforcement of low rate responding (DRL) task, females of the RLA selection line are more efficient in this task than RHA rats (Zeier et al., 1978). This raises the question how measures of impulsivity are related to the coping style and emotionality dimension in the two tier model.

The coping style axis of the two tier model is defined by the way animals respond to a wide variety of environmental challenges, including the display of aggressive behavior in a standard resident-intruder paradigm. Proactive coping animals display high levels of aggressive behavior, whereas reactive animals tend to be non-aggressive and more flexible in unstable environmental conditions (Koolhaas et al., 1999). The concept of proactive coping would predict that aggressive males are better active shock avoiders than non-aggressive males. Indeed, male mice that have been bi-directionally selected for aggression (short and long attack latency; SAL and LAL line respectively) (Van Oortmerssen and Bakker, 1981) show considerable differences in two-way active avoidance behavior. SAL mice show higher levels of avoidances (Benus et al., 1989) and impulsive behavior in several behavioral tests compared to LAL mice (Benus et al., 1990).

Based on these data, we hypothesized that RLA rats will not only be characterized by low levels of active avoidance behavior, but also by low levels of offensive aggressive behavior in a resident-intruder paradigm and low levels of impulsive behavior in an unpredictable operant conditioning paradigm for food reinforcement. On the other hand, we expected that proactive coping RHA rats would show high levels of offensive aggressive behavior and show rigid behavior in an unpredictable operant conditioning paradigm.

To test these hypotheses, we first addressed the question to what extent these selection lines still fulfill their original selection criterion by exposing animals to a two-way active avoidance paradigm. In addition, the level of offensive aggression was determined in a standard resident-intruder paradigm. Performance on an unpredictable (variable interval 15 seconds, VI-15) operant conditioning schedule for food reinforcement was used as a measure for impulsivity. Finally, we determined the response to frustrating non-reward during extinction of lever-press behavior.

MATERIALS AND METHODS

Animals

Breeding pairs of the Roman selection lines (10 of each line) were obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI), University of Geneva, Switzerland. Male RHA rats (n = 12, 384 ± 9 gram) and RLA (n = 12, 427 ± 12 gram) rats used for the experiments were bred under laboratory conditions in our own facilities. Rats were weaned at post-natal day (pnd) 21 and housed in pairs in macrolon type 3 cages until the start of the experiment (~pnd 150).

Animals were housed in temperature-controlled rooms (21 ± 2°C) under a 12 hr light: dark cycle (lights off at 10 am). Water was available *ad libitum* throughout the experiment, food was restricted during operant conditioning tests, but standard lab chow was otherwise available *ad libitum*. Experiments were approved by the Groningen University Committee on Animal Experiments.

Experimental design

First, animals were tested for shuttle-box behavior. Thereafter half of the animals were

tested in a standard resident-intruder paradigm for their level of offensive aggression directly followed by screening for impulsivity levels in an operant conditioning paradigm. The other half was tested for impulsivity first, directly followed by the aggression screening. Behavioral tests took place during the first half of the dark phase.

Shuttle-box behavior

The shuttle-box consisted of two equally sized compartments (24×25×17 cm) with a grid floor, the two compartments were separated by an elastic band at a height of 6 cm. Animals were habituated to the box for 60 seconds prior to each session. The conditioned stimulus (CS) was a light (from a small TL lamp). The unconditioned stimulus (US) commenced 10 seconds after the CS (i.e. US and CS were overlapping) and was a scrambled electric shock of 0.30 mA delivered through the grid floor (maximum 15 seconds). The stimuli were terminated when the animal crossed to the other compartment, with crossings during the CS being considered as active avoidance responses. After an avoidance response or upon termination of the US an inter-trial interval (ITI) of 20 seconds started. Training consisted of 5 sessions of 30 trials, with sessions separated by one day. The number of active avoidances and inter-trial crossings were analyzed.

Aggression screening

Animals were housed in large observation cages (80×55×50 cm) with an oviduct-ligated female (wild-type Groningen rat) for one week to avoid social isolation and facilitate territorial behavior. After one week, the baseline level of aggressive behavior was tested in the resident-intruder test. The female was removed from the test cage prior to testing. During the first three tests an unfamiliar male conspecific (Wistar intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 minutes the intruder was removed. During the fourth test the full range of behaviors was scored during 10 minutes. The frequency and duration of behavioral elements were scored. A total of 12 behavioral acts and postures were scored and grouped in 5 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration* (moving towards, nosing, investigating opponent, anogenital sniffing, crawl over, attempted mount, social groom); 3) *Non-social exploration* (ambulation, rearing, sniffing, scanning, digging); 4) *Inactivity* (sitting, lying, immobile, freezing); 5) *Self-grooming* (washing, shaking, scratching). The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals.

Operant conditioning

Skinner-box equipment (Med Associates Inc., St. Albans, VT) was installed in the home cage (45×30×50 cm) of the animals. Sawdust was used as bedding material. One retractable lever was located besides a food receptacle. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a

computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms.

At the start of the operant conditioning phase, animals were housed in the operant conditioning cages. Normal chow was removed one day prior to operant testing. Animals were tested daily and remained in the operant conditioning cages throughout the experiment. The body weight of animals was gradually decreased to 90% of their free feeding bodyweight. Approximately 3 hours after operant conditioning sessions, additional chow was given.

At first, animals were trained to lever press using a fixed-ratio 1 (FR-1) schedule of reinforcement. During these sessions, each lever press resulted in the delivery of one food pellet into the food receptacle. After one week of FR-1 training, the schedule was changed to a variable-interval 15 (VI-15) schedule for one week. During this schedule, the first lever press resulted in a reward, after which a random refractory period started, lasting between 2 and 32 seconds, during which the animal could press the lever, but did not receive a reward. Animals were trained on the VI-15 schedule until stable performance was reached (23 sessions).

One extinction session was performed after the variable interval training. During extinction, the lever was extended and responding still resulted in activation of the food dispenser, but the food dispenser was disconnected and no pellets were distributed. In this way all secondary cues were maintained.

The result of two animals were discarded, one because of malfunctioning of the operant conditioning cage and one because of extremely unstable performance.

Statistics

Shuttle-box behavior was analyzed using repeated measures ANOVAs. *Post-hoc* analysis and other results were compared using student t-tests. A *p*-value smaller than 0.05 (two tailed) was considered to be statistically significant.

RESULTS

Shuttle-box behavior

The number of avoidance responses was significantly higher in RHA compared to RLA rats ($F_{1,22} = 70.7$, $p < 0.001$) (figure 4.1A). Performance during the first shuttle-box session is shown in figure 4.1B. Performance during the first 7 trials was similar. Thereafter, the number of avoidance responses was significantly higher in RHA rats ($F_{29,638} = 15.0$, $p < 0.001$) . Furthermore, the number of inter-trial crossings (data not shown) showed a similar pattern and was significantly higher in RHA rats compared to RLA rats ($F_{1,22} = 30.8$, $p < 0.001$).

Offensive aggression

The behavioral profile during a ten minutes resident-intruder test is shown in figure 4.2. We did not find a difference between animals that were first tested in the operant condi-

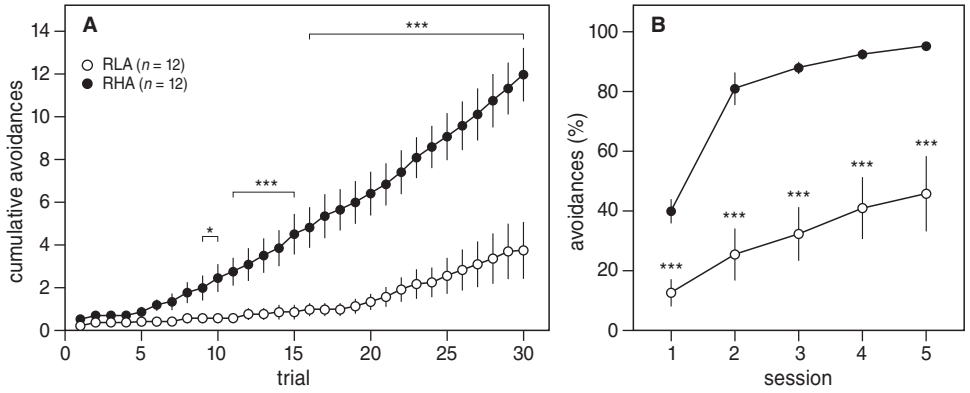

Figure 4.1 (A) The percentage of avoidance responses performed by RLA and RHA rats during shuttle-box training (n = 12 per group). (B) Cumulative avoidance responses during the first shuttle-box session. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

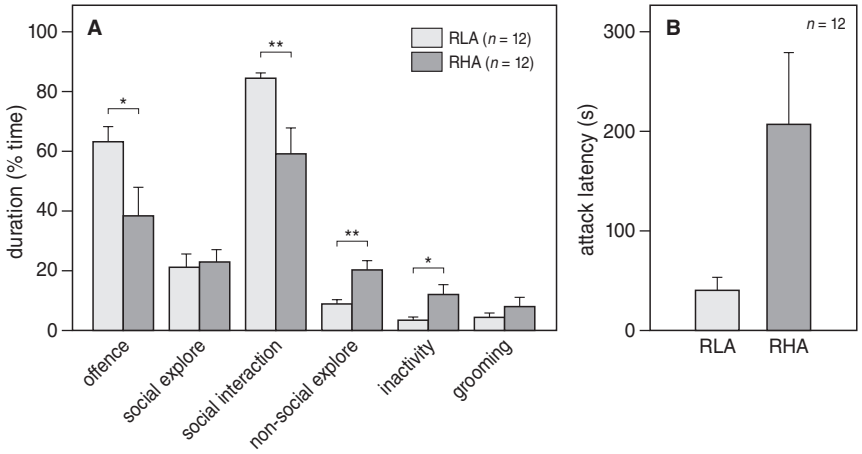

Figure 4.2 (A) Behavior during the resident-intruder test in rats of the Roman selection lines (n = 12 per group). Parameters are expressed as percentage of the total time of the test. (b) Attack latency during the last resident-intruder test. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$.

tioning paradigm and animals that were first tested in the resident-intruder paradigm. Therefore, data of the two batches were combined and analyzed together. The level of offensive aggressive behavior was significantly higher in RLA compared to RHA rats (p=0.03). Furthermore, the amount of time spent on social interaction (p=0.008) was lower in RHA rats. Non-social exploration (p=0.006) and immobility (p=0.02) was significantly higher in RHA rats compared to RLA rats (figure 2A). The attack latency (during the last test) was significantly lower in RLA rats compared to RHA rats (p=0.03) (figure 2B).

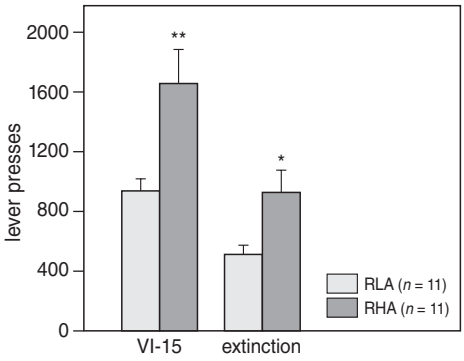

Figure 4.3 Performance on the last VI-15 session of reinforcement and during extinction in RLA and RHA rats ($n = 11$ per group). The data are expressed as the total number of presses ± SEM. $*p < 0.05$, $**p < 0.01$.

Operant behavior

RHA rats performed a significantly higher number of lever presses compared to RLA rats on the last sessions of the FR-1 and the VI-15 schedule of reinforcement ($p = 0.03$ and $p = 0.008$ respectively). Furthermore, the efficiency of lever pressing on the VI-15 schedule (number of obtained rewards divided by the number of lever presses) was higher in RLA rats ($p = 0.003$). However, the number of food receptacle visits did not differ between the two lines on the FR-1 and VI-15 schedule ($p = 0.57$ and $p = 0.07$ respectively). During extinction, RHA rats responded more compared to rats of the RLA line ($p = 0.02$). The number of lever presses during the VI-15 schedule of reinforcement and extinction is shown in figure 4.3. The number of rewards and food receptacle visits during the different operant conditioning schedules are summarized in table 4.1.

Table 4.1 Performance on the different schedules of reinforcements in rats of the Roman selection lines ($n = 11$ per group). All schedules lasted for one hour. The number of rewards obtained during the FR-1 schedule equals the number of lever presses on this schedule. Data are expressed as mean ± SEM. * Indicates a significant line difference.

		RLA	**RHA**
Schedule	Parameter	Mean ± SEM	Mean ± SEM
FR-1	Rewards	261 ± 17	306 ± 9*
	Food receptacle visits	450 ± 46	496 ± 58
VI-15	Rewards	175 ± 3	186 ± 5
	Food receptacle visits	293 ± 26	400 ± 111
Extinction	Food receptacle visits	93 ± 26	86 ± 17

$*p < 0.05$.

DISCUSSION

The present results confirm that avoidance performance in a two-way active avoidance paradigm is different between rats of the Roman selection lines. RHA rats rapidly learned to respond with active avoidance upon presentation of the conditioned stimulus, whereas RLA rats showed poor performance in the shuttle-box task. These results closely resemble the behavioral performance during this task shown by others and are consistent with the original selection criterion for rats of the Roman selection lines (Bignami, 1965; Driscoll, 1986; Escorihuela et al., 1999).

During the first part of the first session, RHA rats and RLA rats perform similarly. Thereafter, RHA rats show more active avoidances than RLA rats. Differences in avoidance behavior are most likely not caused by differences in learning and memory. Despite their low performance in the active avoidance task, RLA rats are superior in an appetitive working memory task (Guenaire and Delacour, 1985; Willig et al., 1991) and perform better in reference memory tasks as well (Willig et al., 1991). A more plausible explanation for differences in avoidance behavior is that RLA rats show higher emotional reactivity and therefore more freezing behavior compared to RHA rats. In addition, the number of inter-trial crossings is higher in RHA rats, which has been shown before by others (Diaz-Moran et al., 2012). The species-specific defense theory of avoidance learning by Bolles states that aversive situations may lead in some animals to defensive reactions such as freezing and in other animals non-defensive reactions such as avoidance are conditioned. This may suggest that a general increase in activity may have been conditioned in RHA rats, whereas freezing (a species-specific defense reaction) has been conditioned in RLA rats (Crawford and Masterson, 1982).

Interestingly, there were large individual differences in the performance of RLA rats. About half of the RLA rats did not show any avoidance acquisition, whereas the other half of the RLA rats acquired avoidance behavior, although they were slower compared to RHA rats. While Roman rats are genetically selected, large inter-individual differences in behavioral performance can still be found (Steimer and Driscoll, 2005). A similar dichotomy in active shock avoidance performance has been found in LAL mice as well (Benus et al., 1989).

The parameters of the shuttle-box procedure were similar to the original article of Bignami (1965). However, instead of 50 trials per session, we used 30 trials on 5 consecutive days and the shock intensity was lower (0.3 mA instead of 1.6 mA). The CS was twice as long (10 seconds) compared to the procedure used by Bignami and only one box was used for the entire experiment. Whereas shuttle-box behavior is often tested in the light phase, we examined behavior in the dark phase. Driscoll showed that the widely divergent behavioral response is maintained during this phase (Driscoll, 1986).

We hypothesized that RHA rats would be characterized by high levels of offensive aggressive behavior. Remarkably, the level of offensive aggression was higher in RLA compared to RHA rats. A previous study performed in our laboratory failed to show statistically significant differences in aggression in Roman rats (Coppens et al., 2012). In rats of the unselected wild-type Groningen strain, which differs widely in the expression of offen-

sive aggressive behavior, we failed to show a relation between aggressive behavior and avoidance behavior as well (Van der Vegt, 2002).

The type of intruders and context used for aggression screening appears to play a major role in the behavioral outcome. We used intruders of a different strain (Wistars). Unpublished data show that RHA rats are more aggressive than RLA rats when intruders of the same line are used (Steimer, personal communication). The level of aggressive behavior in rats of the Roman selection lines has also been tested by others in a shock-induced aggression paradigm. The deficit in two-way active avoidance seen in RLA rats is accompanied by inhibited shock-induced fighting (Driscoll et al., 1980; Siegel et al., 1993). However, offensive aggression is, in terms of motor patterns, adaptive function and neurobiology, different from shock-induced defense-like aggressive behavior (Albert and Walsh, 1982).

High levels of offensive aggression might be the result of diminished behavioral inhibition due to a low anxiety level. Rats that have been genetically selected for differences in anxiety-related behavior on the elevated plus maze (Liebsch et al., 1998) also show different levels of aggressive behavior (Veenema et al., 2007). HAB rats displaying high levels of anxiety-related behavior are less aggressive than their low LAB (low anxiety-related behavior) counterparts (Veenema et al., 2007).

Anxiety-related behavior in the Roman rats may play a role in the display of aggressive behavior as well. However, the findings on the level of anxiety-related behavior are contradictory. Whereas one study shows that RLA rats display less anxiety-related behavior than RHA rats when they are exposed to the elevated plus-maze and the black/white box test (Chaouloff et al., 1994), most other studies show a higher level of anxiety in RLA rats (Driscoll et al., 1998; Escorihuela et al., 1999; Steimer and Driscoll, 2003). We therefore conclude that active avoidance behavior and offensive aggression are two unrelated behavioral constructs.

Whereas anxiety is considered to be the result of high emotional reactivity combined with a reactive coping style, low emotional reactivity combined with a proactive coping style is hypothesized to result in impulsive behavior (Steimer et al., 1997). The amount of lever pressing in the unpredictable operant conditioning task was higher in RHA rats compared to RLA rats. During the extinction session similar differences in performance were found compared to the VI-15 schedule of reinforcement.

In the context of the operant conditioning paradigm, the amount of lever pressing in the unpredictable task was higher in RHA rats compared to RLA rats. During the extinction session similar differences in performance were found compared to the VI-15 schedule of reinforcement. The behavioral performance on the variable interval schedule of reinforcement and during extinction are similar to previous results obtained with Roman rats in our laboratory (Coppens et al., 2012). The results also confirm data obtained in rats of the unselected wild-type Groningen rat strain, in which proactive coping rats are characterized by rigid behavior (Coppens et al., 2011b).

It can be reasoned that differences in responding in the operant conditioning task are caused by differences in activity and not by a difference in impulsivity. However, RHA rats performed similar to RLA rats during the training phase in which a predictable schedule

of reinforcement was used (fixed ratio schedule).

It remains a question to what extent behavior measured in the VI-15 schedule relates to impulsivity. Performance in the VI-15 schedule of reinforcement seems to reflect the ability to respond in a flexible way to cues in the environment. Efficient performance in this paradigm is strongly determined by the capacity of the animal to tune its lever pressing to the cues of the delivery of food pellets.

The low level of behavioral flexibility in RHA rats matches with results obtained by others. RHA rats have been shown to be less capable of inhibiting non-relevant activity under a DRL 20-s schedule of reinforcement. However, the effect was mainly seen in female rats and not in male rats (Zeier et al., 1978). Moreno and colleagues showed that RHA rats have poor inhibitory control in a five-choice serial reaction time task, and show higher choice impulsivity than RLA rats in a delay-discounting paradigm (Moreno et al., 2010).

Taken together, results of the two-way active avoidance task and VI-15 performance in rats of the Roman selection lines fit with the two tier model of coping styles. Unexpectedly, the level of offensive aggression does not match with this model. In a recent review, we proposed that the dimensions of the coping style model should reflect the differential pattern of activation of the underlying neuronal network and the behavioral control function of its components (Coppens et al., 2010). This indicates that it is likely that the neuronal network involved in the regulation of aggressive behavior is different from the neuronal network involved in active avoidance and impulsive behavior.

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CHAPTER 5

Coping style predicts behavioral flexibility but not cardiovascular response during reinforced and extinguished operant behavior in rats

Caroline M. Coppens, Bauke Buwalda, Jaap M. Koolhaas, Sietse F. de Boer

ABSTRACT

This study aims at understanding the relationship between behavioral flexibility and individual stress vulnerability using the wild-type Groningen (WTG) rat. This strain is characterized by a wide and consistent individual variation in coping style in terms of behavior and physiology. Proactive, aggressive males are characterized by a high reactivity of the sympathetic nervous system and behavioral rigidity. Reactive coping, non-aggressive males are more flexible. This study tests the hypothesis that proactive males differ from reactive coping males in their response to an unpredictable environment or a changing environment.

Individual WTG rats were first characterized for their level of aggressive behavior and subsequently subjected to operant conditioning tasks. Behavioral flexibility was tested using a variable interval schedule of reinforcement. Frustrative non-reward, i.e. extinction was used to test the response to a sudden change in an otherwise stable environment. The physiological response during operant conditioning was monitored using radiotelemetry.

The number of responses on a variable interval schedule of reinforcement was positively correlated with aggressive behavior, whereas the efficiency of lever pressing was negatively correlated. During extinction, all animals reduced the number of lever presses and individual differences in the number of responses were less abundant. Furthermore, extinction resulted in a decreased heart rate and blood pressure compared to the response during variable interval training.

Proactive coping is less efficient in an unpredictable environment. Heart rate and blood pressure during both the unpredictable and the uncontrollable operant conditioning sessions seems to reflect mainly the activity of the animal.

INTRODUCTION

It is well known that blood pressure and heart rate responses reflect both activity and emotional status of an organism. Furthermore, physiological response patterns to social and non-social challenges strongly depend on the degree of control over a situation. Unpredictable and uncontrollable situations in general evoke a higher response than predictable and fully controllable situations (Vogel, 1985; Henry et al., 1986).

When control over the environment is challenged (i.e., fight and/or flight effort), the sympathetic adrenomedullary system is activated as well as the hypothalamic pituitary-adrenocortical system (Henry et al., 1986; De Boer et al., 1990; Goldstein, 2010; Buwalda et al., 2012). There are large individual differences in the quality and the quantity of the neuroendocrine response during and after stress and it is thought that the individual behavioral response strategy of animals determines the neuroendocrine outcome.

Individual variability in behavior and underlying physiology is often categorized in distinct personalities or temperaments. Coping styles are the animal equivalent of personality in humans and may be defined as alternative response patterns in reaction to a stressor (Koolhaas et al., 1999). A proactive coping style is characterized by high levels of aggression, acting on the basis of predictions and a high reactivity of the sympathetic nervous system in response to stressors. Whereas reactive coping animals show low levels of aggression, act based on previous experiences and respond with lower reactivity of the sympathetic nervous system compared to proactive animals (Koolhaas et al., 1999; Koolhaas et al., 2010).

Furthermore, proactive animals are considered to form rigid behavior and high levels of impulsive behavior (Benus et al., 1987; Benus et al., 1990; Benus et al., 1991; David et al., 2004; Cervantes and Delville, 2007). Impulsive behavior and behavioral flexibility are often assessed in operant conditioning paradigms, These paradigms allow full experimental control over the stimuli that the animal has to respond to and of the responses they make. In a delayed reward paradigm aggressive male golden hamsters tend to respond more impulsively than low aggressive hamsters as well (David et al., 2004; Cervantes and Delville, 2007). In a recent study we confirmed these findings showing that in an unpredictable food reinforcement task, proactive coping animals respond more impulsive than reactive rats (Coppens et al., in prep.).

In a study by De Boer and colleagues, neuroendocrine responses were measured during a variable interval schedule for food reinforcement . During reinforced responding on this schedule, plasma noradrenaline increased, whereas adrenaline levels did not change and corticosterone declined after an initial response. In the same study, frustrating non-reward (i.e. extinction) was shown to lead to a transient increase in adrenaline, decreased noradrenaline and elevated corticosterone concentrations. In addition, a positive correlation existed between the number of lever responses and the noradrenaline reactivity (De Boer et al., 1990).

Although considerable individual differences exist between individual animals in the behavioral and hormonal response under different operant conditioning situations it is

unknown whether this is related to coping style and reflected in physiological parameters such as heart rate and blood pressure response.

In the current study, we investigated individual differences in behavioral and physiological responses during reinforced and extinguished operant behavior in rats. We used rats of the feral, wild-derived wild-type Groningen (WTG) strain, which are characterized by a wide and consistent individual variation in their level of offensive aggression expressed towards an unfamiliar intruder male, ranging from no aggression at all to very high levels of aggressive behavior.

In WTG rats a positive correlation exists between aggression levels and the noradrenaline and adrenaline response to a stressful event (Sgoifo et al., 1996). This supports the general view that the aggressive, proactive coping male is characterized by a high reactivity of the sympathetic nervous system while baseline levels of adrenaline and noradrenaline are generally similar.

We tested the hypothesis that proactive males are more impulsive and exhibit a higher sympathetic response, as measured by heart rate and blood pressure, to unpredictable and uncontrollable operant conditioning schedules.

MATERIALS AND METHODS

Animals

Adult male Wild-Type Groningen (WTG) rats (*Rattus Norvegicus*; originally wild-trapped animals and bred under conventionalized conditions for over 50 generations in our own facilities) were used. Animals (n = 21) were housed in temperature-controlled rooms (21 ± 2°C) under a 12h reversed light:dark cycle (lights off at 11 am) with water available *ad libitum*. Animals were food restricted to 85–90% of their free-feeding bodyweight. All experiments were approved by the Groningen University committee on Animal Experiments.

Resident-intruder test

Wild-type Groningen rats were characterized for the level of offensive behavior using resident-intruder tests. Animals were housed in large observation cages (80×55×50 cm) with a female (tubally-ligated) for one week to avoid social isolation and facilitate territorial behavior. After one week, the baseline level of aggressive behavior was tested in the resident-intruder test. Thirty minutes prior to testing, females were removed. During the first three tests an unfamiliar Wistar male (intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 minutes the intruder was removed. During the fourth test the full range of behaviors was scored during 10 minutes after the first attack. The frequency and duration of behavioral elements were scored. A total of 12 behavioral acts and postures were scored and grouped in 5 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration* (moving towards, nosing, investigating opponent, ano-

genital sniffing, crawl over, attempted mount, social groom); 3) *Non-social exploration* (ambulation, rearing, sniffing, scanning, digging); 4) *Inactivity* (sitting, lying, immobile, freezing); 5) *Grooming* (washing, shaking, scratching). The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals. For correlations with operant conditioning, we used the time spent on offensive behavior.

Telemetry

Rats were anaesthetized with a mixture of isoflurane (5% to induce and 3% to maintain) and oxygen. For the biotelemetry recordings, a blood pressure transmitter (PA-C40, Data Sciences International, St. Paul, MN, USA) was implanted surgically in the intraperitoneal cavity and attached to the abdominal wall to ensure stabilization. The catheter of the transmitter was inserted into the ventral aorta to measure blood pressure. After surgery, animals were given sufficient time to recover from the surgery. Operant conditioning cages were placed on receiver platforms and data were collected using Dataquest Labpro software. Recordings of heart rate and blood pressure were obtained by sampling segments of 10 seconds every 5 minutes during which animals were left undisturbed in their home cage. Only animals with a reliable signal during all operant conditioning schedules were used ($n = 19$ for heart rate responses and $n = 13$ for blood pressure responses).

The area under the curve between $t = 0$ and $t = 75$ min was calculated using Sigmaplot 11.0.1 software. The average heart rate and blood pressure between $t = -30$ and $t = -10$ was used as baseline.

Operant conditioning apparatus

Operant conditioning equipment (Med Associates Inc., St. Albans, VT) was installed in the home cage (45×30×50 cm) of the animals. Sawdust was used as bedding material. One retractable lever was located next to a food receptacle. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms. The number of lever presses and rewards were collected in 5 minutes intervals.

Operant behavior

At the start of the experiment animals were single housed, normal lab chow was removed and the retractable the lever was extended into the chamber and delivered pellets under a fixed ratio 1 schedule (FR-1). 20 pellets were distributed into the food receptacle and 5 pellets were placed on the lever to facilitate lever pressing. After 24 hr on a FR-1 schedule, the availability of the lever was reduced to one hour per day at the start of the dark phase until the end of the experiment. After one week, animals received seven sessions of reinforcements on a variable interval 15 (range 2–32 sec) schedule (VI-15). Thereafter, an extinction session was performed. During extinction, the food dispenser was disconnected and no pellets were distributed, however, all secondary cues remained.

Statistical analysis

The relationship between offensive behavior, operant conditioning behavior and physiological responses was analyzed by calculating Pearson`s correlation coefficients. Correlations between offensive behavior and physiological parameters were based on the area under the curve of heart rate and blood pressure during the final session of the various operant conditioning schedules. The peak response values on the different reinforcement schedules were compared using a repeated measures ANOVA. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

During a VI-15 schedule of reinforcement and during extinction, the level of offensive aggressive behavior and the number of lever presses was correlated (R = 0.79, $n = 21$, $p < 0.0001$ and R = 0.86, $n = 21$, $p < 0.0001$ respectively). The correlation between offensive aggression and VI-15 performance is shown in figure 5.1.

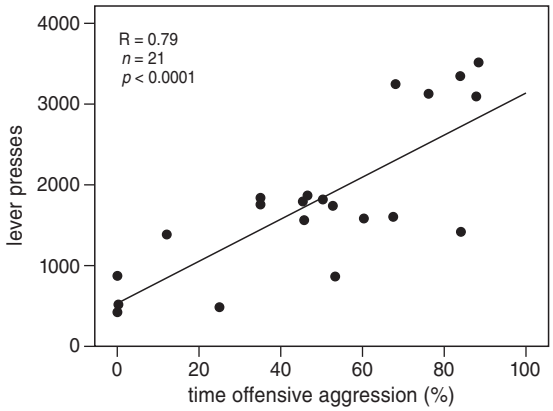

Figure 5.1 Correlation between offensive aggressive behavior and the total number of lever presses on a VI-15 schedule of reinforcement.

Table 5.1 Correlations between time spent on offensive aggression and lever press performance ($n = 21$), heart rate (HR) response ($n = 19$) and blood pressure (BP) response ($n = 13$) response during various operant conditioning schedules.

	Lever presses		Efficiency		AUC HR		AUC BP	
	R	*p*	R	*p*	R	*p*	R	*P*
FR-1	0.37	0.10			0.26	0.29	0.07	0.82
VI-15	0.79	<0.0001	-0.75	<0.0001	0.31	0.20	0.26	0.39
Extinction	0.75	<0.0001			0.21	0.39	-0.09	0.78

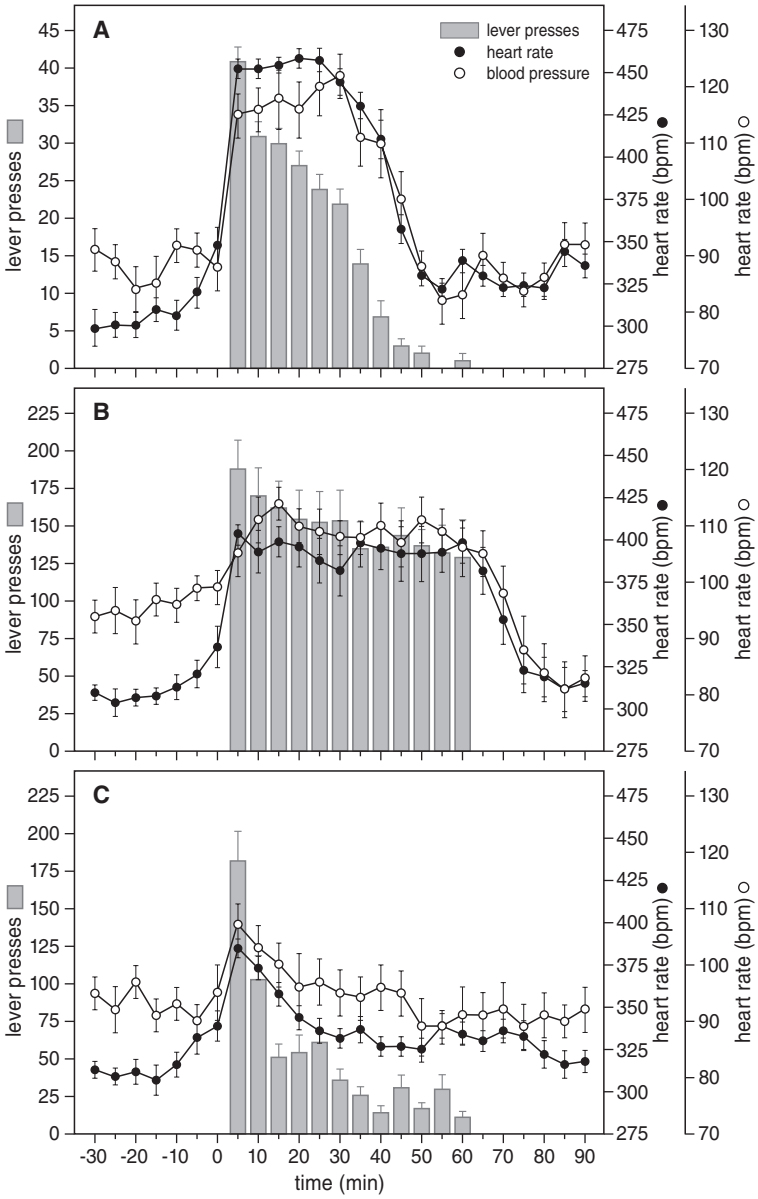

Figure 5.2 Number of lever presses (n = 21), heart rate (n = 19) and blood pressure (n = 13) response during (A) Fixed ratio 1, (B) Variable interval 15 and (C) extinction of lever pressing behavior.

In table 5.1 the correlations between aggression, lever press performance and the physiological response during various operant conditioning schedules is shown. No correlation was found between aggressive behavior and FR-1 performance. Figure 5.2 depicts the heart rate and blood pressure responses on the FR-1, VI-15 and extinction schedule.

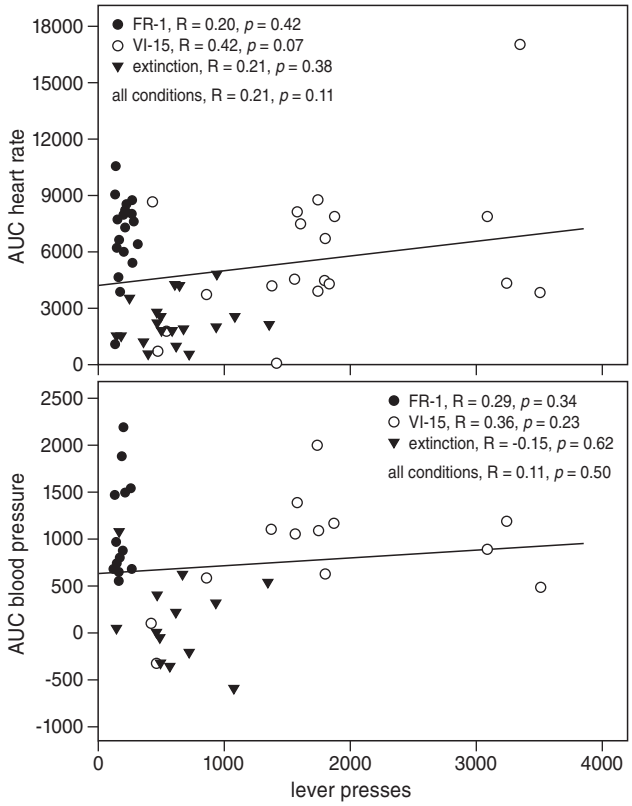

Figure 5.3 Relationship between the number of lever presses on various schedules of reinforcement (FR-1, VI-15 and extinction) and the (A) heart rate response (n = 19) (B) Blood pressure response (n = 13).

The heart rate and blood pressure rise during lever pressing and decrease when the number of lever presses is reduced to baseline levels when the lever is retracted at the end of the operant conditioning session.

The number of lever presses on the various reinforcement schedules did not correlate with the heart rate and blood pressure responses (figure 5.3). No correlation was found between aggression and heart rate and blood pressure responses (table 5.1).

The correlation between peak heart rate response and aggression and lever pressing behavior is given in table 5.2. The peak heart rate responses were significantly different during the FR-1, VI-15 and extinction schedule ($F_{2,36} = 54.10$, $p < 0.0001$). The peak value during the VI-15 schedule was correlated with the number of lever presses on this schedule (R = 0.51, n = 19, p=0.03).

In table 5.3 the peak blood pressure response and correlations with aggression and lever pressing is given. The peak blood pressure response was significantly different between the three different conditions ($F_{2,24} = 26.34$, $p < 0.0001$). There is a correlation between the peak blood pressure response and the number of lever presses on the FR-1 schedule (R = 0.56, n = 13, p = 0.05).

Table 5.2 Peak heart rate response (bpm, $n = 19$) during different schedules of reinforcement and the correlation of the peak response with the level of aggression and number of lever presses.

	Mean ± SEM	$R_{aggr.}$	$p_{aggr.}$	$R_{presses}$	$P_{presses}$
FR-1	480 ± 5	0.10	0.68	0.32	0.19
VI-15	429 ± 9	0.36	0.13	0.51	0.03
Extinction	400 ± 4	0.26	0.29	0.17	0.49

Table 5.3 Peak blood pressure response (mmHg, $n = 13$) during different schedules of reinforcement and the correlation of the peak response with the level of aggression and number of lever presses.

	Mean ± SEM	$R_{aggr.}$	$p_{aggr.}$	$R_{presses}$	$P_{presses}$
FR-1	134 ± 3	-0.09	0.78	0.56	0.05
VI-15	120 ± 3	0.25	0.41	0.15	0.63
Extinction	113 ± 4	0.06	0.85	0.39	0.19

DISCUSSION

The present results show that proactive coping, high-aggressive animals exhibit more impulsive behavior as indicated by a higher number of lever presses during a variable interval schedule of food reinforcement. Hence, they are less efficient in an unpredictable environment. Secondly, heart rate and blood pressure responses are temporally associated, but not quantitatively correlated, with lever pressing performance.

Regarding the relationship between impulsivity and aggression, results are consistent with measures of impulsivity using operant conditioning in hamsters (David et al., 2004; Cervantes and Delville, 2007) and rigid performance of proactive coping mice and pigs in maze tasks (Benus et al., 1987; Benus et al., 1990; Benus et al., 1991; Bolhuis et al., 2004).

The efficiency of lever pressing on a variable interval schedule is correlated to the level of offensive aggressive behavior: high aggressive animals are less efficient compared to low aggressive animals. These results confirm that reactive coping animals are doing better under variable and unpredictable environmental conditions, whereas proactive coping animals are more adapted to stable environmental conditions. Individual variation in coping with different environmental conditions leads to differences in fitness and protects the species against fluctuations in the environment (Dingemanse et al., 2004; Sih et al., 2004a).

We hypothesized that the different strategies of animals on various operant conditioning schedules is reflected in the physiological response as well. The heart rate and blood pressure of the animals during different operant conditioning situations clearly corresponds to the behavioral performance in time. When lever pressing activity of the animals

was highest, heart rate and blood pressure levels were at the highest levels as well. The quantity of the response (the exact number of lever presses) did not determine the physiological response.

The peak heart rate and blood pressure was significantly reduced between the various operant conditioning schedules. Although the number of lever presses on the VI-15 schedule was highest, the peak heart rate and blood pressure were lower compared to the FR-1 schedule, during which the number of lever presses is much lower. This effect might be induced by the food restriction we used, which in general reduces the blood pressure (Wright et al., 1981; Walford et al., 1992). The number of lever presses on the FR-1 schedule was not correlated with aggressive behavior. However, the peak blood pressure response did correlate with the number on this schedule. A similar correlation was found between the number of lever presses on the VI-15 schedule and the peak heart rate response. This might indicate that the difference in peak heart rate and blood pressure is dependent on the activity of the animal (lever presses), but not dependent on the coping style of animals.

Because proactive coping is generally characterized by an active response to environmental challenges, we hypothesized a positive correlation between behavioral and physiological (i.e. heart rate and blood pressure) response. However, we did not find a relationship the level of offensive aggression and the physiological response during the various operant conditioning paradigms. In general, the sympathetic nervous activity is considered to be a direct reflection of the metabolic and cardiovascular demands required for physical activity (Koolhaas et al., 2010). The current experimental setup did not involve a high metabolic and cardiovascular load and we therefore might not find a relationship between the coping style of animals and the physiological response during operant conditioning.
The spontaneously hypertensive-rat (SHR) is the most frequently used animal model for attention-deficit hyperactivity disorder (ADHD), which is characterized by high levels of impulsive behavior (Sagvolden, 2000). It seems that a tendency to show high blood pressure responses is accompanied by high levels of impulsive behavior. However, a high blood pressure response does not always predict high levels of impulsive behavior. In a study by Wickens and colleagues, F-2 hybrids from cross-bred genetically hypertensive (GH) and Wistar rats tested on a fixed-interval followed by an extinction component (FI-EXT) schedule showed remarkable individual differences in responding. Lever press performance on this schedule did not correlated with blood pressure in these rats. They concluded that the pattern of responding during operant conditioning is not genetically linked with hypertension (Wickens et al., 2004).

The number of lever presses during extinction decreased relatively rapidly in the current experiment. The extinction session started at the beginning of the operant conditioning session and no increase in heart rate and blood pressure response compared to reinforced sessions was found. Adrenocortical activity has been shown to be sustained and even further elevated in response to extinction of lever pressing (Coover et al., 1971; Goldman et al., 1973; Davis et al., 1976; Dantzer et al., 1980; Coe et al., 1983; Coover et al., 1986). Other experiments show that when behavior is first reinforced and after 10 minutes extinguished the behavioral outcome and the stress hormones are showing more

extreme profiles compared to behavior that is extinguished from the beginning of a session (De Boer et al., 1990). With respect to the predictability and controllability of the extinction session, it would be interesting to see if behavior that is first reinforced and within the same session extinguished leads to an increased heart rate and blood pressure responses. In conclusion, offensive aggression is correlated with behavioral flexibility measured in a VI-15 schedule of reinforcement. However, the cardiovascular response during different operant conditioning paradigms is not related to the coping style of rats.

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CHAPTER 6

Adolescent social defeat disturbs adult aggression-related impulsivity in wild-type rats

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ABSTRACT

Adolescence is generally considered as a developmental period during which adverse social experiences may have lasting consequences in terms of an increased vulnerability to affective disorders. This study aimed at determining the individual susceptibility to adolescent social stress using a rat model. In adult male rats, coping style is an important determinant of individual stress vulnerability. Coping style reflects individual differences in behavior that are consistent over time and across situations. However, it is unclear when coping style characteristics exactly stabilize during development and to what extent they can change due to adverse experiences.

We used rats of the Wild-type Groningen strain, which are characterized by a broad variation in adult coping style. Behavioral performance in an unpredictable operant conditioning paradigm for food reinforcement was used to study the development of individual differentiation between adolescence and adulthood. We expected that adolescent rats show low behavioral inhibition and that operant performance in adolescence predicts adult coping style. Furthermore, we hypothesized that experience of social defeat in adolescence results in heightened adult aggression and impulsivity levels.

In contrast to our expectation, behavioral performance in an unpredictable lever pressing was similar in adolescence and adulthood. In addition, adolescent performance did not predict adult coping style. Adolescent social defeat did not lead to a difference in the average adult level of aggression and impulsivity, but the significant correlation between offensive aggression and impulsivity found in control animals was not present in animals defeated during adolescence.

Submitted for publication

INTRODUCTION

Adolescence is generally considered as a developmental period that is essential for the development of adult social skills. Several studies indicate that adverse social experiences in this phase of life may have lasting consequences in terms of an increased vulnerability to affective disorders (Van Os and Jones, 1999; Gladstone et al., 2006; Arseneault et al., 2010).

In human adolescents, an important source of social stress is bullying. Although bullying may have serious and lasting consequences for some adolescents, others do not seem to suffer from any negative consequences after experiencing bullying (Ortega et al., 2009; Rudolph et al., 2011). This clearly indicates that certain individuals are more vulnerable to bullying whereas others seem to be resilient. This is consistent with the well-recognized phenomenon that individual differences in sensitivity to stress are a critical factor in the development of psychopathologies both in humans and in animals (Schmidt et al., 2008; Kotov et al., 2010). However, the behavioral characteristics of individuals that are susceptible to bullying and to its consequences are poorly understood.

This study aims at an experimental approach of the individual susceptibility to adolescent social stress using a rat model. In adult male rats, coping style is an important determinant of individual stress vulnerability. Coping styles can be defined as suits of correlated behaviors that are consistent over time and across situations (Koolhaas et al., 1999). However, it is unclear when coping style characteristics exactly stabilize during development and to what extent they can change due to adverse experiences. Studies on the development and maturation of coping style between adolescence and adulthood are scarce.

There is some information on the development of aggressive behavior, which is an important component of adult coping style (Koolhaas et al., 1999; David et al., 2004; Cervantes and Delville, 2007). In hamsters, puberty is marked by a transition of play fighting into adult aggressive behavior. Whereas play fighting is characterized by attacks that do not lead to functional consequences, adult attacks are more serious and meant to defend the territory and food resources of an animal (Pellis and Pellis, 1998; Wommack and Delville, 2003). Repeated social stress during adolescence in male golden hamsters was shown to accelerate the onset of adult-like aggressive behavior and the level of attack frequency in early adulthood is higher in stressed animals (Delville et al., 2003; Wommack and Delville, 2003; Wommack and Delville, 2007). Furthermore, social isolation during development has been shown to lead to heightened offensive aggression levels in adulthood in mice (Terranova et al., 1998).

Delville and colleagues suggested that the prefrontal cortex is involved in both aggressive and impulsive behavior (Cervantes and Delville, 2007). The prefrontal cortex in particular is undergoing major changes during adolescence (Giedd et al., 1999; Spear, 2000; Andersen, 2003). Furthermore, the prefrontal cortex has been shown to be involved in the development of adult behavioral skills by facilitating juvenile play (Pellis et al., 1992).

We hypothesize that prefrontal cortex functioning is affected by adolescent social stress and focused on levels of adult offensive aggressive behavior and impulsivity as measures

for prefrontal cortex functioning (Mobini et al., 2002; Blair, 2004; David et al., 2004; Dalley et al., 2008; Siever, 2008; Coppens et al., 2010). In general, adolescents act more impulsively and engage in riskier behavior than adults (Kelly and Deadwyler, 2003; Laviola et al., 2003; Andrzejewski et al., 2011). Therefore, we expected that adolescents show higher impulsivity than adults, with high rank-order stability indicating that adult aspects of impulsivity may be reliably predicted from levels seen in adolescence.

In the adult male rat, aggressive behavior is strongly correlated with behavioral performance in a variable interval schedule operant conditioning paradigm. Low aggressive males are extremely efficient in tuning their operant responding to the variable interval schedule (Coppens et al., 2011b). Since the capacity to learn an operant conditioning paradigm is largely independent of age, the performance in a variable interval schedule can be used as an early indicator of individual variation in juveniles and a predictor of adult coping style.

We used rats of the Wild-Type Groningen (WTG) strain to study the development of individual differentiation between adolescence and adulthood. Rats of the WTG strain show a broad variation in the level of offensive aggressive behavior towards an unfamiliar intruder, ranging from no aggression at all to very high levels of aggressive behavior (De Boer et al., 2003). Furthermore, high-aggressive animals do not perform very efficient in an unpredictable operant conditioning paradigm (variable-interval 15 s) compared to low-aggressive animals (Coppens et al., 2011b).

To induce social stress in the adolescent male rats, we used the social defeat paradigm. Björkvist suggested that the social defeat paradigm may be an ecological valid animal model to study the consequences of bullying (Bjorkqvist, 2001). Social defeat induces long lasting behavioral and neurochemical changes in adult male rats (Koolhaas et al., 1997; Bjorkqvist, 2001). Based on similar studies performed in hamsters, in which accelerated onset of adult aggression levels were found after social stress in adolescence (Wommack and Delville, 2003; Wommack et al., 2003), we hypothesized that social defeat in adolescence may result in heightened adult aggression and enhanced impulsivity.

METHODS

Ethics statement

Experiments were approved by the Groningen University Committee on Animal Experiments (DEC5538E) and we certify that the international standards for animal protection (European Communities Council Directive of 24 November 1986, 86/609/EEC) have been followed.

Animals

Male Wild-Type Groningen (WTG) rats (*Rattus Norvegicus*), originally wild-trapped animals and consequently bred under laboratory conditions for over 50 generations in our own facilities, were used as experimental subjects. They were weaned at pnd 21 and housed in non-sibling (randomly mixed) groups of three animals in standard macrolon

cages (type 4). Companion females were tubally ligated (to prevent pregnancy while maintaining hormonal tone and sexual receptivity) under O_2-isoflurane anaesthesia in adulthood. After surgery females were allowed to fully recover while being socially housed with four females per cage (macrolon type 4). Cages with the animals were placed in temperature-controlled rooms (21 ±2°C) under a 12h light-dark cycle (lights off at 12 am). Water was available *ad libitum* throughout the experiment. Food was restricted to maintain animals at ~90% of normal bodyweight during operant conditioning tests, but otherwise lab chow (Hope Farms, RMH-B knaagdier korrel, Arie Block Diervoeding, Woerden, NL) was available *ad libitum*.

Experimental design

During early adolescence (pnd 28–44), impulsivity levels were measured by lever press performance on an unpredictable food-reinforced operant conditioning paradigm (variable-interval 15 schedule of reinforcement) for all subjects. Thereafter, animals were divided in a control (n = 21) and a defeat group (n = 21) matched on their lever-pressing performances in the operant conditioning task.

Subjects in the defeat group were defeated during late adolescence (pnd 45–57). At an age of 4 months, all subjects were tested for their level of offensive aggression using a resident-intruder paradigm. One day after the resident-intruder tests, operant conditioning for food reinforcement started to test the level of adult impulsivity.

Operant conditioning during adolescence

Skinner-box equipment (Med Associates Inc., St. Albans, VT) was installed in clear perspex cages (45×30×50 cm) with sawdust as bedding material. One retractable lever was located next to a food receptacle; the position of the lever was counterbalanced between cages. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms.

Normal chow was removed on pnd 28 and food pellets were provided in the cage to allow the animals to get used to the pellets prior to operant testing. From pnd 29 to pnd 44 animals were tested daily for one hour at the start of the dark phase in the skinner-box setup. Animals were placed in the operant conditioning cages 10 minutes prior to the onset of the dark phase. One hour after the end of operant testing each animal received an additional amount of chow sufficient to maintain animals at ~90% of normal bodyweight (measured by an age-matched *ad libitum* fed control group). All animals were returned to their home cage one hour after feeding.

At first, animals were trained to lever press using a fixed-ratio 1 (FR-1) schedule of reinforcement. During these sessions, each lever press resulted in delivering of one food pellet into the food receptacle. After one week of FR-1 training (7 sessions), the schedule was changed to a variable-interval 15 (VI-15) schedule until pnd 44 (stable performance, 9 sessions). During this schedule, the first lever press resulted in a reward, after which a

random refractory period started, lasting between 2 and 32 seconds, during which the animal could press the lever, but did not receive a reward. The total number of lever presses emitted during the last VI-15 session was used for the statistical analysis.

Adolescent social defeat

The resident-intruder paradigm was used for the social defeat. A similar procedure has been used in previous experiments in our laboratory (Vidal et al., 2007). Resident rats of the WTG strain were housed with a tubally-ligated female in large observation cages (80×55×50 cm) to facilitate territorial aggression. Shortly (10 min) before the conflict, females were removed from the cage of the residents. Residents were trained to attack naïve intruders and only those with attack latencies shorter than 2 minutes were used for the experiment. By using animals with a more or less similar readiness to attack and aggression level, we tried to avoid variation in attack intensity.

Adolescent rats were subjected to social defeat at pnd 45 and 48 with direct physical contact with the aggressive resident for 10 minutes, thereafter animals were placed in a wire mesh cage (31×15×15 cm) for an additional 50 minutes in the cage of the resident. In this way, animals were protected from further attacks and injury, but remained in full visual, auditory and olfactory contact with the resident. This period of psychosocial stress is known to be highly stressful (Tornatzky and Miczek, 1994). All animals showed clear submissive behavior, 2 animals were placed in the wire mesh cage after 7 minutes to avoid serious bite wounds. On pnd 51, 54 and 57 animals were only psychosocially stressed by placing them in the residents' cage for 15 minutes. Defeats took place during the first half of the dark phase at approximately the same time each trial. Control animals were placed in a clean cage at corresponding days and times compared to defeat animals. Control animals were housed in groups of three animals until the start of the offensive aggression screening. Defeated animals were solitary housed after the first defeat for the rest of the experiment.

Resident-intruder test

Experimental subjects were tested for aggressive behavior at ~pnd 120. Animals were housed in large observation cages (80×55×50 cm) with a tubally-ligated WTG female to avoid social isolation and facilitate territorial behavior. After one week, the level of aggressive behavior was tested daily in the resident-intruder test on four consecutive days. Females were removed from the test cage prior to testing. During the first three tests an unfamiliar male con-specific (intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 minutes the intruder was removed. During the fourth test the full range of behaviors was scored during 10 minutes. The frequency and duration of behavioral elements were scored. All behavioral acts and postures were scored and grouped in 6 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration* (moving towards, nosing, investigating opponent, ano-genital sniffing, crawl over, attempted mount, social grooming); 3) *Social interaction* (sum of total offense and social

exploration); 4) *Non-social exploration* (ambulation, rearing); 5) *Immobility* (sitting, lying, immobile, freezing); 6) Grooming. The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals. For correlations with operant conditioning, we used the percentage of time spent on offensive behaviors.

Operant conditioning during adulthood

Operant conditioning training was conducted daily and done comparable to the operant conditioning during adolescence. During the entire operant conditioning phase, animals were housed in the operant conditioning cages. Food was removed and 5 gram of standard lab chow was provided in the cage. The next day, the retractable lever was extended into the chamber during the first hour of the dark phase and delivered pellets under a FR-1. After three sessions on a FR-1 schedule, animals received reinforcement sessions according to a VI-15 schedule until stable performance for at least three days (7 sessions). The total number of lever presses during the last VI-15 session was used for the comparison with adolescent operant performance. The difference between the number of lever presses on the first and the last day of adult VI-15 was used for correlations with offensive aggressive behavior. The bodyweight of animals was gradually decreased to 90% of their free feeding bodyweight. Approximately 3 hours after operant conditioning sessions additional chow was given.

Statistical analysis

Group data of the offensive aggression test and operant conditioning data are expressed as averages with standard error of the mean and analysed using a two-tailed student t-test. Differences between adolescent and adult operant conditioning behavior were analysed using a two-way repeated-measures ANOVA. To analyse correlations between various behaviors (i.e. adolescent and adult operant behavior, offensive aggression and attack latency time) two-tailed Pearson correlation coefficients were used. Comparing correlations was done by converting the correlation coefficient to a z-score and comparing this to a normal distribution. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Operant behavior

Figure 6.1A displays the lever press performance (corrected for bodyweight) on the VI-15 schedule of reinforcement during adolescence and adulthood. The number of lever presses did not differ between adolescence and adulthood ($F_{1,39} = 0.19$, $p = 0.66$). No difference was found in the number of lever presses between control and defeat animals ($F_{1,39} = 0.21$, $p = 0.65$) and no interaction effect was found ($F_{1,39} = 1.03$, $p = 0.32$). No correlation between lever presses between adolescence and adulthood was found (Fig. 6.1B Control: $R = 0.09$, $p = 0.70$; Fig 6.1C Defeat: $R = 0.14$ $p = 0.54$)

The number of obtained rewards was higher in adulthood ($F_{1,40} = 167.0$, $p < 0.0001$). No difference was found between control and defeat animals in the total number of

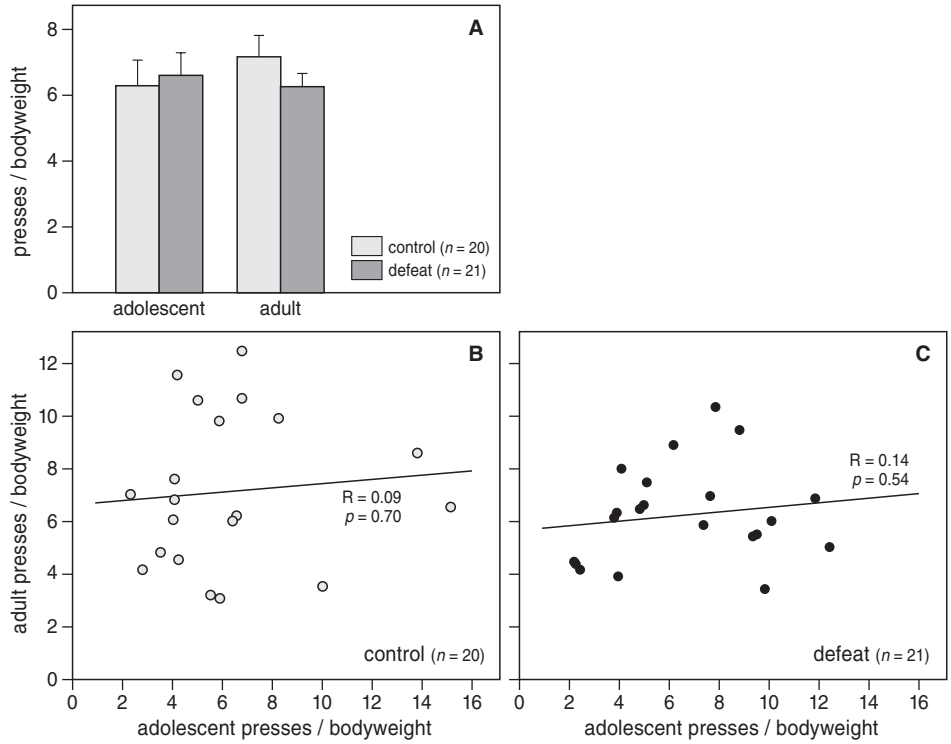

Figure 6.1 (A) Total number of lever presses corrected for bodyweight during adolescence and adulthood on a VI-15 schedule of reinforcement. The data points of 4 non-aggressive animals are overlapping. No difference was found between control and defeat animals. However, adult animals showed a higher level of responding compared to adolescent animals. (B) Correlation between adolescent and adult lever presses (corrected for bodyweight) on a VI-15 schedule of reinforcement in control animals. (C) Correlation between adolescent and adult lever presses (corrected for bodyweight) in defeat animals. No correlation between adolescent and adult lever press performance was found in control and defeat animals.

rewards ($F_{1,39} = 1.09$, $p = 0.30$). Also, no correlation between the number of rewards obtained during adolescence and adulthood was found (Control: R = 0.22, $p = 0.35$; Defeat: R = 0.00, $p = 0.99$). One animal was excluded from the analysis, because it performed an unusual high number of lever presses compared to all other animals in adolescence.

Offensive aggression

Figure 6.2A shows the ethogram of the behavior during a 10 min social interaction in the resident-intruder test. There was no difference between control and defeated animals in any of the recorded behaviors.

The attack latencies (Fig. 6.2B) between control (202 ± 47 s) and defeated animals (136 ± 35 s) did not differ ($p = 0.27$, $n = 21$). However, a correlation between attack latency and offensive aggression was found in control animals (R = -0.68, $n = 21$, $p =$

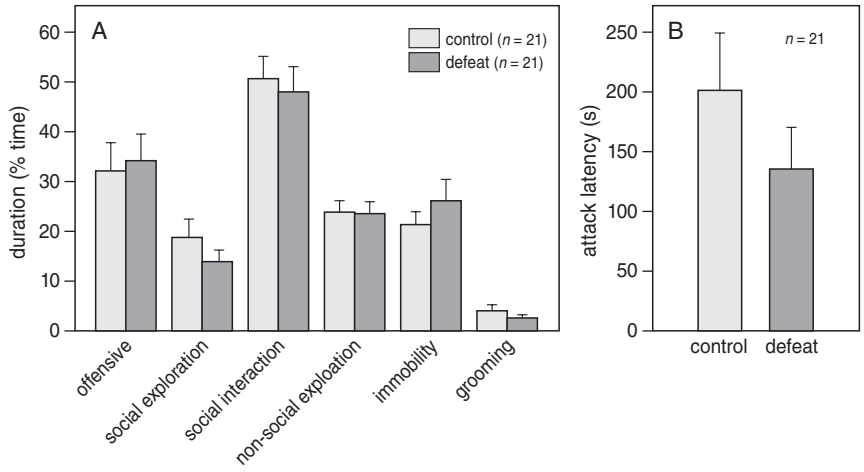

Figure 6.2 (A) Ethogram of adult resident-intruder test for control (n = 21) and defeat animals (n = 21). None of the behavioral parameters is significantly different between the two groups. (B) Attack latency during the last resident-intruder test.

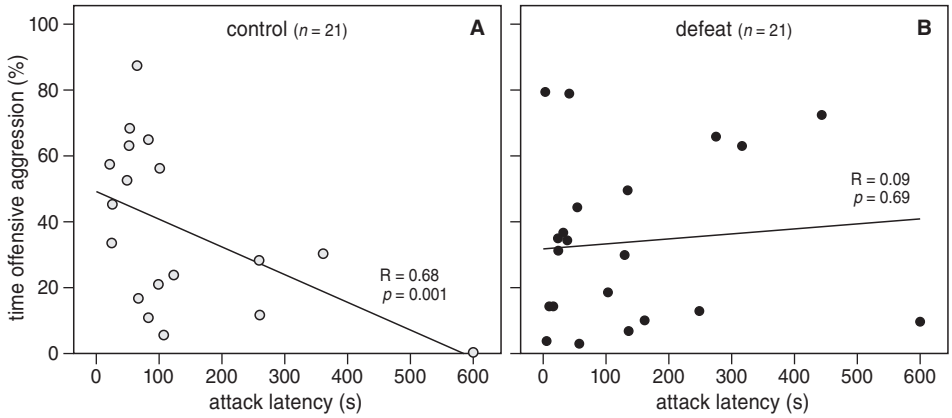

Figure 6.3 Correlation between attack latency and time spent on aggressive behavior during the resident-intruder test in (A) control and (B) defeat animals. The attack latency was correlated with the time spent on aggressive behavior in control animals, but not in defeat animals.

0.001), whereas no correlation was found in defeated animals (R = 0.09, n = 21, p = 0.69) (Fig. 6.3). There was a significant difference between the correlations of control and defeat animals (z=2.18, p = 0.03).

Correlation between operant behavior and offensive aggression

Adolescent VI-15 performance was not correlated with adult offensive aggression (Control: R = -0.41, n = 21, p = 0.07; Defeat: R = -0.18, n = 21, p = 0.43). The correla-

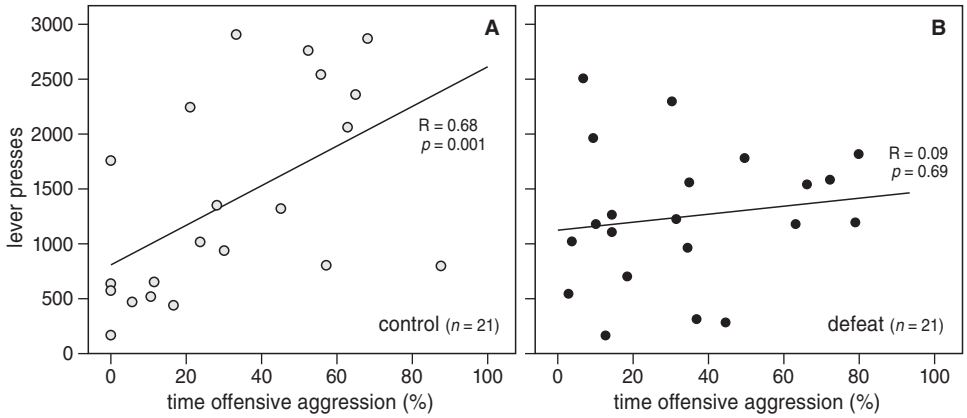

Figure 6.4 (A) Performance on a VI-15 schedule is correlated with offensive aggression in control animals ($R = 0.53$, $n = 21$, $p = 0.01$). (B) Performance on a VI-15 schedule of reinforcement is not correlated with offensive aggression in defeat animals ($R = 0.15$, $n = 21$, $p = 0.53$). The number of lever presses in the correlations is corrected for baseline performance.

tion between offensive aggression and performance on a VI-15 in adulthood is depicted in figure 6.4. For control animals there is a clear positive correlation between VI-15 performance and offensive aggression ($R = 0.53$, $n = 21$, $p = 0.01$), whereas in defeat animals no correlation was found ($R = 0.15$, $n = 21$, $p = 0.53$). The correlations of control and defeat animals did not differ significantly ($z = 1.32$, $p = 0.19$). The number of lever presses used in the correlation was corrected for baseline differences.

DISCUSSION

The aim of this study was to investigate individual susceptibility to adolescent social stress and the development of individual differentiation between adolescence and adulthood. We show that performance on an unpredictable operant conditioning schedule for food reinforcement during adolescence neither predicts adult aggressive behavior nor adult performance in this paradigm. This means that there is no rank-order stability of this component of adult coping style between adolescence and adulthood.

Although behavioral traits are considered to be stable over a certain period of time, the evidence for stability between adolescence and adulthood is scarce (Stamps and Groothuis, 2010). There are only a few studies showing predictive measures for adult coping style. Bolhuis and colleagues have shown that piglets that struggle a lot in the back-test appear to adopt a proactive coping style in other tests as adults as well (Bolhuis et al., 2004).

However, based on the current data we propose that coping style characteristics are still not fixed during adolescence. Human studies show that personality matures during adolescence and that a plateau level in personality profile similarity is reached in late

adolescence (Roberts et al., 2001; Blonigen et al., 2008; Klimstra et al., 2009). It would be interesting to confirm this by determining the level of impulsive behavior in late adolescence (e.g., pnd 45-60) in rats as well.

Adolescence is generally characterized by low behavioral inhibition overtly expressed in more impulsive acts, failure to consider long-term consequences and engaging in riskier behavior (Spear, 2000; Kelley et al., 2004; Andrzejewski et al., 2011). Indeed, when adolescent and adult rats are tested as independent experimental groups, adolescent animals show lower levels of behavioral inhibition and self-control (Andrzejewski et al., 2011). Adolescent mice show increased elevated impulsivity levels compared to adult mice (Adriani and Laviola, 2003). Our data, employing a within group comparison, do not support these findings. In the present experiment a direct comparison between adolescent and adult performance may not be justified, since all adult animals had experience with the operant conditioning schedule during adolescence.

With regard to aggression, puberty is marked by a transition of play fighting into adult patterns of aggressive behavior. Whereas play fighting is characterized by attacks that do not lead to functional consequences, adult attacks are more serious and expressed to acquire or defend social status, territory or resources (e.g., food) of an animal (Pellis and Pellis, 1998; Wommack et al., 2003). Delville and colleagues studied the consequences of adolescent social stress on the development of aggression in male golden hamsters. They showed that the onset of adult-like agonistic responses is accelerated after repeated social stress and the attack frequency is higher in early adulthood in socially stressed animals. However, the average level of aggression in adulthood did not differ between socially stressed and control animals (Wommack et al., 2003). Our results confirm this finding, since we showed that the group level of offensive behavior did not differ between control and defeat animals at postnatal day 120.

The experience of social defeat during adolescence neither affected the average level of adult aggressive behavior, nor did it change operant conditioning performance on the VI-15 schedule of reinforcement. However, the correlation between adult offensive behavior and VI-15 performance that is repeatedly found in control animals is not present in defeated animals. Moreover, the expected negative correlation between attack latency and time spent on aggressive behavior as usually observed in non-stressed control groups of our animals (De Boer et al., 2003) was also not observed in the defeated animals. This might be an additional indication for a disturbed relationship between aggression and behavioral impulsivity in the defeated subjects.

Impulsivity, as a trait-characteristic, is closely related to and an important factor for initiation of aggression, both in animals and in humans (Rudebeck et al., 2007; García-Forero et al., 2009). It remains a question to what extent behavior measured in the VI-15 schedule relates to impulsivity. However, the highly significant correlation between offensive aggression and VI-15 performance suggests that this indeed reflects aspects of impulsivity, which is consistent with the coping style model. In general, high levels of impulsivity and aggressive behavior are components of a proactive coping style (Koolhaas et al., 1999; David et al., 2004; Cervantes and Delville, 2007; Coppens et al., 2011b). Performance in the VI-15 schedule of reinforcement seems to reflect behavioral flexibility as well. Efficient

performance in this paradigm is strongly determined by the capacity of the animal to tune its lever pressing to the cues of the delivery of food pellets.

It is remarkable that social defeat also disturbs the relationship between attack latency and the amount of time spent on offensive behavior. This confirms that individuals are not equally vulnerable to the consequences of adolescent social stress and social defeat seems to disturb the normal development of coping style characteristics.

Our data emphasize the importance of social experiences in the development of coping styles. Adult coping styles are likely to be the result of a strong gene environment interaction (Caspi et al., 2005; Stamps and Groothuis, 2010; Coppens et al., 2011a). Steimer and Driscoll (2003) came to a similar conclusion in rats of the Roman selection lines, which is a genetic model for differences in coping styles. Some behavioral differences are already visible shortly after birth, but the full pattern of behaviors stabilizes only after puberty and is most clear during adulthood (Steimer and Driscoll, 2003).

A possibly confounding factor in the current experiment is the solitary housing of adolescent animals after the experience of social defeat. In adulthood, social isolation after defeat appears to be a necessary, but in itself not sufficient, factor for inducing the long-term effects of defeat. Social housing after defeat has been shown to reduce the impact of social defeat (Ruis et al., 1999; De Jong et al., 2005). Although isolation of animals during weeks 4 and 5 of age causes a reduction of adult social activity, isolation after this period does not influence the pattern of social activity in adulthood (Hol et al., 1999). We isolated animals after the peak in play behavior (Meaney and Stewart, 1981; Hol et al., 1999) and therefore did not expect alterations in social behavior caused by the isolation per se. We decided to use group housed control animals to avoid disturbance of the normal development (Van den Berg et al., 1999).

It is tempting to consider the possibility that the disrupted correlation between aggression and behavioral flexibility reflects altered prefrontal cortex functioning since this brain structure is associated with both aggression and impulsivity (Coppens et al., 2010). The prefrontal cortex is undergoing major structural and functional changes during adolescence (Spear, 2000; Andersen, 2003) and exposure to periods of intense stress causes structural remodelling of neurons within the prefrontal cortex which leads to executive dysfunction in rodents (McEwen, 2005; Holmes and Wellman, 2009). The causal involvement of prefrontal cortex remodelling by adolescent social defeat in aggression and behavioral flexibility during adulthood is the focus of our further research.

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CHAPTER 7

Impulsivity and aggressive behavior in Roman high and low avoidance rats: baseline differences and adolescent social stress induced changes

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ABSTRACT

Adverse and stressful experiences during adolescence are often of a social nature. The social defeat model in rats is used as an animal model for bullying in humans. Usually large individual differences in response to social defeat are found. The personality type that is mostly affected and the underlying mechanisms are unknown. We used male rats of the Roman selection lines to test whether social defeat (between postnatal day 45 and 57) followed by social isolation has a different impact in animals with divergent levels of emotional reactivity and coping style. The level of offensive aggression, impulsivity and performance during frustrating non-reward (extinction) were used as measures for the adult coping style of animals. Impulsivity was measured by performance on an unpredictable operant conditioning schedule (variable interval-15, VI-15).

This study demonstrates that the adult, baseline level of impulsivity is higher in Roman high avoidance (RHA) rats. RHA rats showed a higher number of lever presses compared to Roman low avoidance (RLA) rats on a VI-15 schedule. The level of offensive aggression did not differ between the two lines. Surprisingly, a tendency towards more offensive aggression in RLA rats was found.

Social stress during adolescence disturbed the normal development of adult personality, mostly in RHA rats. RHA rats that were defeated during adolescence reduced the number of lever presses on the VI-15 schedule of reinforcement and were more persistent during a session of frustrating non-reward. However, we did not find an effect of social defeat on performance during extinction. A tendency towards increased attack latencies after social defeat in adolescence was found. The time spent on offensive aggression was unaffected by social defeat.

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INTRODUCTION

Adolescence is a period in life during which there is a steep increase in the incidence of psychopathologies such as depression (Hankin et al., 1998), anxiety disorders (Bernstein et al., 1996) as well as substance abuse and addiction (McNeill, 1991; Laviola et al., 1999; O'Malley and Johnston, 2002; Schneider, 2008). Among many developmental factors that contribute to this early onset of psychopathology, exposure to uncontrollable stress during adolescence may be particular relevant (Van Os and Jones, 1999; Gladstone et al., 2006).

An important source of social stress in adolescents is being the victim of bullying and harassment. Bullying is associated with the development of severe symptoms of mental health problems, which are frequently long-lasting (Kumpulainen, 2008; Arseneault et al., 2010). However, large individual differences in the consequences of bullying are found. Some victims do not suffer from any negative consequences after experiencing bullying, whereas others report severe negative emotions (Ortega et al., 2009). This is consistent with the general notion that individual differences in sensitivity to stress and coping capacity are critical in the development of psychopathologies both in humans and in animals (Schmidt et al., 2008; Kotov et al., 2010). The mechanisms underlying those individual differences in susceptibility to the consequences of bullying are poorly understood.

In the current study, social defeat followed by social isolation was used as stressor in adolescent rats. This paradigm can be regarded as an animal model of bullying in humans (Bjorkqvist, 2001). Social defeat in adolescence has been shown to induce long-term behavioral changes, such as increased anxiety (Tsoory et al., 2007; Vidal et al., 2007; Toth et al., 2008a; Vidal et al., 2011a, c), a reduced behavioral response to amphetamine in adulthood (Burke et al., 2010), and an accelerated onset of adult-like agonistic behavior (Delville et al., 2003; Wommack et al., 2003; Wommack and Delville, 2007).

Similar to the consequences of bullying in humans, there are large individual differences in the response to social defeat in animals as well. Some animals appear to be more resistant to social stress than others. The emotional response to social stress might be a key factor in determining the long-term consequences of social defeat stress. Therefore, we used rats of the Roman selection lines to test whether social defeat has a different impact in animals with distinct levels of emotional reactivity and sensitivity to stressors (Steimer et al., 1997).

The proactive coping Roman high avoidance (RHA) rats have been selected for a high level of active avoidance in a two-way shuttle box task, whereas Roman low avoidance (RLA) rats show a more passive response in this task (Bignami, 1965; Steimer and Driscoll, 2005). RLA rats show more pronounced emotional responses and increased activation of the hypothalamus-pituitary-adrenal (HPA) axis in response to stress compared to RHA rats (Aubry et al., 1995; Steimer et al., 2007). On the other hand, RHA rats tend to be more impulsive (Steimer and Driscoll, 2003; Moreno et al., 2010) during operant behavioral conditioning tasks for food reinforcement (Moreno et al., 2010).

The current experiment was aimed at testing the baseline differences in impulsivity and aggression in RHA and RLA rats and the adult consequences of social stress during adolescence on impulsivity and aggression. We hypothesized higher baseline levels of offensive

aggressive behavior and impulsivity in RHA rats compared to RLA rats. Based on the increased anxiety levels after adolescent social stress observed by others (Vidal et al., 2007; McCormick et al., 2008), we expected a general decrease in impulsive and aggressive behavior in defeated rats of both strains. To test these hypotheses, we used a standard resident-intruder paradigm and an unpredictable (variable interval 15, VI-15) operant conditioning schedule for food reinforcement. Furthermore, extinction of lever-press behavior was used to determine performance during frustrating non-reward. We expected socially defeated animals to be less persistent during the extinction session compared to control animals.

MATERIALS AND METHODS

Animals

Breeding pairs of the Roman selection lines were obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI), University of Geneva, Switzerland. Roman High Avoidance rats and Roman Low Avoidance rats were bred under laboratory conditions in our own facilities. Rats were weaned at post-natal day (pnd) 21 and housed in groups of four until the start of the experiment.

Animals were housed in temperature-controlled rooms (21 ±2°C) under a 12 hr light: dark cycle (lights off at 12 am). Water was available *ad libitum* throughout the experiment, food was restricted during operant conditioning tests, but was otherwise available *ad libitum*. Experiments were approved by the Groningen University Committee on Animal Experiments.

Experimental design

Rats of the Roman selection lines were defeated during adolescence (pnd 45–57). At an age of 4 months, animals were tested for the level of offensive aggression using a resident-intruder paradigm. After the resident-intruder paradigm, impulsive behavior was measured by an unpredictable operant conditioning food reinforcement schedule and extinction of lever pressing behavior.

Adolescent social defeat

The resident-intruder paradigm was used for the social defeat. A similar procedure has been used in previous experiments in our laboratory [15]. Resident rats of the Wild-type Groningen (WTG) strain were housed with a tubally-ligated female in large observation cages (80×55×50 cm) to facilitate territorial aggression. Before the social conflict, females were removed from the cage of the residents. Residents were trained to rapidly and consistently attack naïve intruders and only those with attack latencies shorter than 2 minutes were used for the experiment. By using animals with a more or less similar readiness to attack, we tried to avoid variation in attack intensity.

Adolescent rats were subjected to social defeat at pnd 45 and 48 with direct physical contact for 10 minutes, thereafter animals were placed in a wire mesh cage

(31×15×15 cm) for 50 minutes in the cage of the resident. In this way, animals were protected from further attacks and injury, but remained in visual, auditory and olfactory contact with the resident. This period of psychosocial stress is known to be highly adverse (Tornatzky and Miczek, 1994). On pnd 51, 54 and 57 animals were psychosocially stressed by placing them in the residents` cage for 15 minutes. Control animals were placed in a clean cage at corresponding days and times compared to defeat animals. Defeated animals were solitary housed after the first defeat for the rest of the experiment.

Resident-intruder test

The experimental animals were tested for aggressive behavior between pnd 120 and pnd 130. Animals were housed in large observation cages (80×55×50 cm) with an oviduct-ligated female of the corresponding (selection) line for one week to avoid social isolation and facilitate territorial behavior. After one week, the baseline level of aggressive behavior was tested in the resident-intruder test. Females were removed from the test cage prior to testing. During the first three tests an unfamiliar male conspecific (Wistar intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 minutes the intruder was removed. During the fourth test the full range of behaviors was scored during 10 minutes. The frequency and duration of behavioral elements were scored. A total of 10 behavioral acts and postures were scored and grouped in 5 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration*; 3) *Non-social exploration* (ambulation, rearing); 4) *Inactivity* 5) *Self-grooming*. The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals.

Operant conditioning

Skinner-box equipment (Med Associates Inc., St. Albans, VT) was installed in the home cage (45×30×50 cm) of animals with sawdust as bedding material. By testing animals in their home cage, we avoided handling and transport prior to testing and the behavior could be recorded undisturbed (Koot et al., 2009). One retractable lever was located next to a food receptacle. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms. At the start of the operant conditioning phase, animals were housed in the operant conditioning cages.

Normal chow was removed one day prior to operant testing. Animals were tested daily and remained in the operant conditioning cages throughout the experiment.

At first, animals were trained to lever press using a fixed-ratio 1 (FR-1) schedule of reinforcement. During these sessions, each lever press resulted in delivering of on food pellet into the food receptacle. After one week of FR-1 training, the schedule was changed to a variable-interval 15 (VI-15) schedule for one week. During this schedule, the first lever press resulted in a reward, after which a random refractory period started, lasting between

2 and 32 seconds, during which the animal could press the lever, but did not receive a reward. At the end of the VI-15 schedule an extinction session was done during which the tubing of the food delivery was disconnected from the cage, but all secondary cues were maintained. This session lasted for one hour, at the same time as reinforced sessions took place.

The body weight of animals was gradually decreased to 90% of their free feeding body-weight. Approximately 3 hours after operant conditioning sessions additional chow was given.

Statistical analysis

Differences in groups of the Roman selection lines were determined using a two-way ANOVA. The selection line and experimental group were used as between subject factors. Student t-tests were used as post-hoc test. Distribution of lever presses over time was analyzed using repeated measures ANOVA and Tukey *post-hoc* test. Group data are expressed as averages with standard error of the mean. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Offensive aggression

The results of the resident-intruder test are given in table 7.1. There is a trend towards a significant increase in average attack latency in defeat animals ($F_{1,27} = 3.34$, $p = 0.08$) and interaction between strain and treatment ($F_{1,27} = 2.82$, $p = 0.11$). The total level of offensive aggression tended to be higher in RLA rats ($F_{1,27} = 3.74$, $p = 0.06$) compared to RHA rats, no interaction between strain and adolescent social defeat was found

Table 7.1 Behavior during resident-intruder test at pnd ~120 in rats of the Roman selection lines (n = 7–8 per group). Data are expressed as mean ± SEM. Attack latencies are in seconds, the other parameters are expressed as percentage of the total time of the test.

	RLA		RHA	
	Control	Defeat	Control	Defeat
Attack latency (s)	249 ± 58	257 ± 71	147 ± 41	357 ± 64
Offensive aggression	45 ± 12	43 ±11	24 ± 5	27 ± 9
Social exploration	17 ± 6	25 ± 6	13 ± 2	18 ± 3
Non-social exploration	20 ± 4[a]	16 ± 4[a]	27 ± 3	30 ± 6
Inactivity	13 ± 5	12 ± 5	14 ± 3	14 ± 3
Grooming	5 ± 1	5 ± 2[b]	22 ± 3[c]	12 ± 3[b]

[a] Indicates a significant line difference $p < 0.05$
[b] Indicates a significant effect of defeat $p < 0.05$
[c] Indicates a significant interaction between line and defeat $p < 0.05$

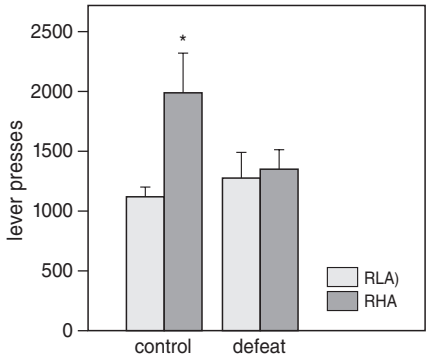

Figure 7.1 Performance on a VI-15 schedule in RLA (n = 7–8) and RHA (n = 8) rats. The data are expressed as the total number of presses ± SEM during the last one hour VI-15 session. RHA rats performed a significantly higher number of lever presses compared to RLA rats ($F_{1,27}$ = 4,43, p = 0.05). Furthermore, there is a trend towards an interaction between strain and treatment ($F_{1,27}$ = 3.16, p = 0.09).

($F_{1,27}$ = 0.09, p = 0.76). RLA rats spent more time on social interaction ($F_{1,27}$ = 10.14, p = 0.004), but less time on non-social exploration ($F_{1,27}$ = 6.04, p = 0.02). A treatment and interaction effect was found in the time spent on grooming ($F_{1,27}$ = 5.63, p = 0.03 and $F_{1,27}$ = 5.31, p = 0.03 respectively), RHA control rats spent more time on grooming compared to the other groups. No effect was found on social exploration and immobility.

Operant behavior

During the fixed-ratio schedule of reinforcement, a difference in the number of lever presses was found between RLA and RHA rats ($F_{1,27}$ = 6.96, p = 0.01). However, no significant interaction was found between strain and treatment ($F_{1,27}$ = 0.55, p = 0.47).

RHA rats performed a significantly higher number of lever presses compared to RLA rats on a variable interval schedule of reinforcement ($F_{1,27}$ = 4.43, p = 0.05). This difference is mainly caused by a difference in the number of lever presses in the control animals (p = 0.03). Furthermore, there is a trend towards a significant interaction effect between strain and treatment ($F_{1,27}$ = 3.16, p = 0.09) (figure 7.1). The number of rewards on the VI-15 schedule did not differ between the two strains ($F_{1,27}$ = 0.45, p = 0.45) and there was no treatment effect ($F_{1,27}$ = 2.10, p = 0.16) on the number of obtained rewards.

During extinction, both control and defeated RHA rats performed significantly more lever presses compared to RLA rats ($F_{1,27}$ = 15.19, p = 0.001). The distribution of lever presses over time is depicted in figure 7.2. There is a significant interaction effect of time, line and treatment ($F_{3,81}$ = 3,76, p = 0.01), the post-hoc test revealed a significant difference between RLA control and RHA control at 15 minutes (p = 0.01). At 30 minutes and 45 minutes, there was a significant difference between RLA defeated and RHA defeated animals (p = 0.01 and p = 0.03). There were no significant differences in the last interval of 15 minutes. The number of rewards and food receptacle visits during the different operant conditioning schedules are summarized in table 7.2.

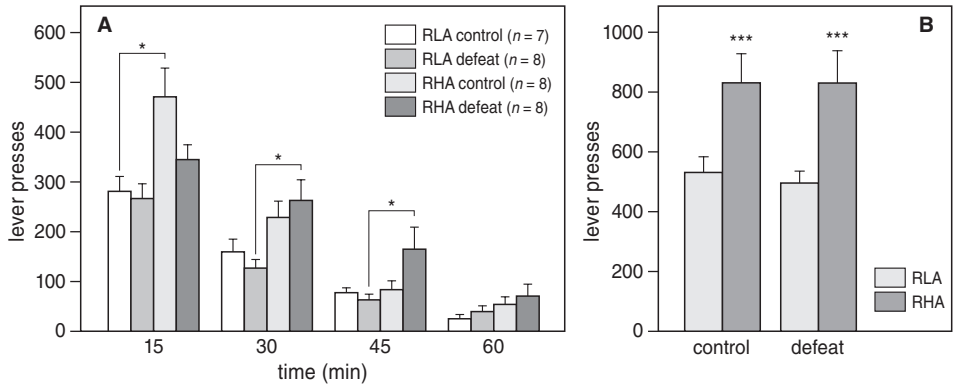

Figure 7.2 (A) The total number of lever presses during an one hour extinction session, ***indicates a significant difference between RHA and RLA animals, $p < 0.001$. Data are expressed as mean ± SEM. (B) The number of lever presses distributed over time (in blocks of 15 min) during 1 h extinction of operant responding. Data are expressed as mean ± SEM. There is a significant interaction between strain, treatment and time ($F_{3,81} = 3.76$, $p < 0.01$) *$p < 0.05$.

Table 7.2 Performance on the different schedules of reinforcement in rats of the Roman selection lines (n = 7–8 per group). All schedules lasted for 1 h. The number of rewards obtained during the FR-1 schedule equals the number of lever presses on this schedule. Data are expressed as mean ± SEM.

		RLA		RHA	
Schedule	Parameter	Control	Defeat	Control	Defeat
FR-1	Rewards	288 ± 16*	294 ± 15*	339 ± 12	322 ± 16
	Food visits	609 ± 81	485 ± 38	519 ± 48	550 ± 106
VI-15	Rewards	175 ± 4	176 ± 4	189 ± 4	171 ± 9
	Food visits	291 ± 40*	261 ± 41*	520 ± 87	356 ± 60
Extinction	Food visits	103 ± 38	61 ± 9	90 ± 11	64 ± 13

*$p < 0.05$

DISCUSSION

This study confirms the finding that RHA rats are more impulsive than RLA rats (Steimer and Driscoll, 2003; Moreno et al., 2010) and shows that social stress experienced during adolescence has long-lasting effects on adult behavior in RHA rats, but not in RLA rats. The baseline differences in impulsivity correspond with a study performed by Moreno and colleagues in which RHA rats have been shown to be more impulsive compared to RLA rats in the delay-discounting paradigm and the five-choice serial reaction time (5-CSRT) task (Moreno et al., 2010). There is a tendency to reduced lever pressing in defeated RHA rats compared to their respective control animals. This may be caused by increased levels

of anxiety (McCormick et al., 2008; Vidal et al., 2011c) and therefore increased inhibitory control induced by the social defeat in adolescence. In RLA rats, the level of impulsivity was not affected by social defeat.

During extinction, the level of responding was lower in all animals compared to reinforced sessions of the variable interval schedule. No difference was found between control and defeated animals neither in RHA nor in RLA rats. However, the distribution of lever presses over time during extinction was significantly affected. In the first time interval a pattern comparable to performance during the reinforced VI-15 schedule was visible, with RHA control animals pressing more compared to both RLA groups. Thereafter, the number of lever presses reduced in all groups. However, defeated RHA rats appeared to be more persistent and less flexible behaviorally than the other groups of animals, they showed more lever presses during the second and the third block of fifteen minutes.

The attack latency of animals can also be regarded as a measure of impulsivity or inhibitory control, since increased attack latencies indicate an inhibition of the tendency to start an aggressive encounter. RHA rats show a tendency to shorter attack latencies, whereas the attack latency of RHA rats is increased due to social defeat during adolescence. The attack latency of RLA rats was not affected by social defeat. Surprisingly, the level of offensive aggression is only slightly higher in RLA rats compared to RHA rats.

Based on the two-tier model in which emotionality and coping style are used as two independent trait characteristics (Steimer et al., 1997; Koolhaas et al., 2007; Koolhaas et al., 2010), we expected RLA rats to be less aggressive than RHA rats. In this model, RHA rats are proactive coping, low emotional animals, whereas RLA rats are reactive coping, high emotional animals. Driscoll and colleagues showed that RHA rats display higher levels of shock-induced aggression compared to RLA rats (Driscoll et al., 1980; Siegel et al., 1993). The inconsistency of our aggression results might be due to the fact that we measured offensive aggression, which in terms of motor patterns, adaptive function and neurobiology is different from shock-induced defensive-like aggressive behavior.

In previous studies in our laboratory, social defeat in rats of the Roman selection lines during adulthood did not induce major differences between the two lines in the long-term consequences (reduced activity, reduced body temperature, reduced open field behavior) of the defeat (Meerlo et al., 1997). This is in contrast with the results of the current study in which adolescent social defeat leads to a differential effect on adult behavior in RHA and RLA rats.

One of the confounding factors in the current experiment may be that the social defeat procedure included individual housing after social defeat. Social isolation appears to be an important factor in the long-term effects of defeat since social housing has been shown to reduce the impact of social defeat (Ruis et al., 1999; De Jong et al., 2005).

The results confirm the data obtained in rats of the wild-type Groningen strain, in which proactive coping rats are characterized by high levels of offensive aggression and high impulsivity levels, whereas low-aggressive animals show low levels of impulsive behavior. In this strain, social defeat during adolescence disturbed this relationship between aggression and impulsivity, which indicates that social stress during this period affects the normal development of personality characteristics (Coppens et al. in prep.).

The underlying neurobiological substrate mediating the long-term effects of social defeat on adult impulsive behavior most likely involves the prefrontal cortex. The relatively late development of this brain structure may be altered by social defeat during adolescence. It has been shown that the prefrontal cortex is undergoing major structural reorganization during adolescence (Kalsbeek et al., 1988; Andersen et al., 2000; Andersen, 2003; Crews et al., 2007; Tseng and O'Donnell, 2007; Cressman et al., 2010). For example, the density of prefrontal cortex derived axon terminals decreases significantly between adolescence and adulthood (Cressman et al., 2010). Also, dopamine D1 and D2 receptors are overproduced prior to puberty and pruned back to adult levels thereafter in the prefrontal cortex (Gelbard et al., 1989; Teicher et al., 1995; Andersen et al., 2000).

Therefore, social defeat might have an impact on these developmental changes and induce altered prefrontal cortex functioning. Indeed, levels of impulsivity (Mobini et al., 2002; Dalley et al., 2008) and aggression (Blair, 2004; Siever, 2008) are strongly related to prefrontal cortex functioning (Coppens et al., 2010). Moreover, stress has been shown to affect behavioral processes involving the prefrontal cortex (Delville et al., 2003; Wommack and Delville, 2003; Wommack et al., 2003; Wommack and Delville, 2007). Several studies show that RHA rats are most susceptible to stress induced changes in prefrontal cortex functioning. D`Angio and colleagues found that prefrontal cortex functioning is affected by stressful environmental stimuli in RHA rats, but not in the hyperemotional RLA rats. Extracellular DOPAC levels are increased after tail pinch, immobilization and forced locomotion in RHA rats (D'Angio et al., 1988).

Furthermore, fear-related behaviors are associated with increased dopamine output in the medial prefrontal cortex of RHA, but not RLA, rats (Giorgi et al., 2003). These data support the general view that the RHA male is more susceptible to stressors, both during adolescence and adulthood. Whether the prefrontal cortex is causally involved in the consequences of social defeat during adolescence on adult coping style needs further research. In summary, this study confirms that RHA rats are characterized by enhanced levels of impulsive-like behavior and that only in RHA rats adolescent social defeat stress induces long-lasting (i.e., decreasing) consequences on adult levels of impulsivity and aggressive behavior.

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CHAPTER 8

Social defeat during adolescence and adulthood differentially induce BDNF-regulated immediate early genes

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ABSTRACT

Stressful life events generally enhance the vulnerability for the development of human psychopathologies such as anxiety disorders and depression. The incidence rates of adult mental disorders steeply rises during adolescence in parallel with a structural and functional reorganization of the neural circuitry underlying stress reactivity. However, the mechanisms underlying susceptibility to stress and manifestation of mental disorders during adolescence are little understood. We hypothesized that heightened sensitivity to stress during adolescence reflects age-dependent differences in the expression of activity-dependent genes involved in synaptic plasticity. Therefore, we compared the effect of social stress during adolescence with social stress in adulthood on the expression of a panel of genes linked to induction of long-term potentiation (LTP) and brain-derived neurotrophic factor (BDNF) signaling. We show that social defeat during adolescence and adulthood differentially regulates expression of the immediate early genes BDNF, Arc, Carp, and Tieg1, as measured by qPCR in tissue lysates from prefrontal cortex, nucleus accumbens, and hippocampus. In the hippocampus, mRNA levels for all four genes were robustly elevated following social defeat in adolescence, whereas none were induced by defeat in adulthood. The relationship to coping style was also examined using adult reactive and proactive coping rats. Gene expression levels of reactive and proactive animals were similar in the prefrontal cortex and hippocampus. However, a trend toward a differential expression of BDNF and Arc mRNA in the nucleus accumbens was detected. BDNF mRNA was increased in the nucleus accumbens of proactive defeated animals, whereas the expression level in reactive defeated animals was comparable to control animals. The results demonstrate striking differences in immediate early gene expression in response to social defeat in adolescent and adult rats.

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INTRODUCTION

Stressful life events generally enhance the vulnerability for the development of human psychopathologies such as anxiety disorders and depression. The majority of adult mental disorders have antecedents and precursors in adolescence. From age 15 incident rates steeply rise for anxiety disorders (Bernstein et al., 1996), depressive disorders (Hankin et al., 1998) as well as delinquency (Landsheer and `t Hart, 1999) and substance abuse and dependence (WHO International Consortium in Psychiatric Epidemiology, 2000).

Adolescence is a period of dynamic (re)organization and formation of the neural circuitry underlying stress reactivity (Andersen, 2003; Romeo and McEwen, 2006). For example, the density of prefrontal cortex derived axon terminals decreases significantly between adolescence and adulthood (Cressman et al., 2010). A pre-adolescent increase in cortical gray matter is followed by a post-adolescent decrease (Giedd et al., 1999). Also, dopamine D1 and D2 receptors are overproduced prior to puberty and pruned back to adult levels thereafter in the striatum and prefrontal cortex (Gelbard et al., 1989; Teicher et al., 1995; Andersen et al., 2000). Therefore, stress during this period may have enduring consequences on mental health later in life via its effect on this process of structural and functional reorganization. However, the mechanisms underlying susceptibility to stress and manifestation of mental disorders during adolescence remain little understood.

We hypothesized that heightened sensitivity to stress during adolescence could reflect age-dependent differences in the expression of genes required for activity-dependent synaptic plasticity, and possibly, cognitive adaptation to stress. Brain-derived neurotrophic factor (BDNF) is among the major regulators of synaptic homeostasis, activity-dependent gene expression, and synaptic plasticity in the adult mammalian brain (Bramham and Messaoudi, 2005; Greenberg et al., 2009; Minichiello, 2009). Behavioral stress in animals is frequently correlated with decreased BDNF expression in the hippocampus and neocortex, while deletion of the BDNF gene is associated with heightened aggression to conspecifics, anxiety, and learning deficits, without affecting depression-like behavior in standard tests (Duman and Monteggia, 2006; Feder et al., 2009; Ito et al., 2011). Treatment with antidepressant drugs triggers enhanced BDNF gene expression and signaling that is required for the restorative behavioral effects in animal models of depression (Alme et al., 2007; Rantamaki et al., 2007; Adachi et al., 2008).

In the dentate gyrus, expression of the immediate early gene Arc (activity-regulated cytoskeleton associated protein) is required for formation of stable, transcription-dependent LTP induced by brief intrahippocampal infusion of BDNF or by brief high-frequency stimulation of the perforant pathway (Messaoudi et al., 2007; Bramham et al., 2010). Wibrand et al. (2006) further identified a panel of genes that are robustly co-upregulated with Arc in dentate granule cells during both forms of LTP. These genes were subsequently found to be differentially regulated in brain-region specific manner following chronic antidepressant treatment in rats (Alme et al., 2007).

The social environment is an important source of stress (conflicts and tension) in everyday life of humans. To induce social stress in rats, we used the social defeat paradigm. Björkvist suggested that the social defeat paradigm is an ecological valid model to study

the consequences of social stress, victimization and social subjugation (Bjorkqvist, 2001). Social defeat has been shown to induce long-lasting behavioral, physiological and neurobiological changes. These include changes in social anxiety, heart rate, body temperature, activity as well as structural and functional changes in various brain neurocircuitries of rats (Meerlo et al., 1999; Buwalda et al., 2005). Therefore, we used social defeat on two consecutive days as a stressor in our experimental animals, which has been shown to induce reliable long-lasting effects on behavior and physiology (Meerlo et al., 1996a).

We hypothesized that age-dependent difference in stress reactivity could reflect differences in the induction of BDNF-regulated and synaptic plasticity-linked immediate early genes. Following exposure to social defeat on two consecutive days, mRNA expression of BDNF (exon IV), Arc, Carp and Tieg1 directly after the second defeat was determined by qPCR in tissue lysates obtained from prefrontal cortex, hippocampus, and nucleus accumbens.

The BDNF, Arc, Carp and Tieg1 genes are strongly upregulated during BDNF-LTP in the dentate gyrus of the hippocampus (Wibrand et al., 2006). Gene expression of BDNF exon IV (i.e. exon III prior to (Aid et al., 2007)) was determined, since this exon is regulated as an immediate early gene and shows a peak level one hour after stimulation (Lauterborn et al., 1996). Carp (calcium/calmodulin dependent protein kinase (CaMK)-related peptide), also known as ANIA-4 (Berke et al., 1998) is an alternative splice variant of the Doublecortin-Like Kinase (DCLK) gene. Tieg1 (Transforming Growth Factor-β inducible early gene) is a member of the specificity protein/Kruppel-like factor (SP/KLF) family of zinc finger transcription factors (Subramaniam et al., 1995; Suske et al., 2005) and enhances TGF-_-dependent gene expression (Johnsen et al., 2002).

The prefrontal cortex was chosen because of its major structural reorganization during adolescence (Kalsbeek et al., 1988) and its important role in emotional regulation (Quirk and Beer, 2006) and aggressive behavior (Blair, 2004; Siever, 2008). The prefrontal cortex is significantly involved in modulation of social behavior and in control of mood and motivational drive, functions that are important components of the personality of an individual (Miller, 2000; Miller and Cohen, 2001). Social defeat has been shown to have a major impact on hippocampal structure and functioning (Buwalda et al., 2005; Artola et al., 2006). In addition, we selected the nucleus accumbens because the ventral tegmental area-nucleus accumbens (VTA-NAcc) pathway has been shown to play a major role in the difference in resilience to social defeat (Berton et al., 2006; Krishnan et al., 2007).

In addition to age-dependent vulnerability to stress, humans exhibit large individual variations in vulnerability to stress-induced disorders and variability in measures of temperament and personality may largely predict this disease risk. Accordingly, variability in behaviorally relevant brain circuit functions due to differences in activity-dependent gene expression and synaptic plasticity may represent one of the causal factors determining the vulnerability to disease. In animals, behavioral differentiation in terms of coping style (personality) reflect trait characteristics that are stable over time (Koolhaas et al., 1999) and these characteristics are strongly correlated with a differentiation in the underlying neurobiological mechanisms (Veenema and Neumann, 2007; Koolhaas et al., 2010). For example, high levels of aggressive behavior are generally associated with low levels of

brain serotonin and its metabolite 5-HIAA (De Boer et al., 2010; Koolhaas et al., 2010). Recent evidence suggests differences in molecular mechanisms of synaptic plasticity as well. For instance, non-aggressive, reactive coping male mice show a higher structural neuronal plasticity (Veenema and Neumann, 2007) and a higher hippocampal and prefrontal cortex expression of neuronal plasticity related genes (Feldker et al., 2003).

To examine a possible link between individual differences in coping to social stress and expression of plasticity-linked genes, adult male wild-type Groningen (WTG) rats were individually characterized for their coping style using their displayed aggressiveness in a resident-intruder paradigm. Rats of this strain differ widely in the level of offensive aggression expressed towards an unfamiliar intruder male, ranging from no aggression at all (reactive coping) to very high levels of intense aggressive behavior (proactive coping). It has been shown that this broad individual variation in aggressiveness can be considered more generally as a variation in actively coping with environmental challenges (De Boer et al., 2003).

MATERIALS AND METHODS

Animals

Adolescent and adult male Wild-type Groningen (WTG) rats (*Rattus Norvegicus*; originally wild-trapped animals and bred under laboratory conditions for over 50 generations in our own facilities) were used. Animals were weaned at postnatal day 28 and housed in groups until the start of the experiment.

All animals were housed in temperature-controlled rooms (21 ±2°C) under a 12h light:dark cycle (lights off at 1 pm) with food and water available *ad libitum*. Experiments were approved by the Groningen University Committee on Animal Experiments.

Characterization of adult animals

Adult animals were screened for the level of offensive aggressive behavior in a standard resident-intruder paradigm at an age of approximately 120 days. Animals were housed in large observation cages (80×55×40 cm) with a sterilized female (oviduct-ligated) for one week to avoid social isolation and facilitate territorial behavior. After one week, the baseline level of aggressive behavior was tested in the resident-intruder test in the first half of the dark phase.

Before testing, the female was removed from the cage. During the first three tests an unfamiliar male conspecific (intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 minutes the intruder was removed.

During the fourth test the full range of behaviors was scored during 10 minutes. The frequency and duration of behavioral elements were scored. A total of 12 behavioral acts and postures were scored and grouped in 5 behavioral categories: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture); 2) *Social exploration* (moving

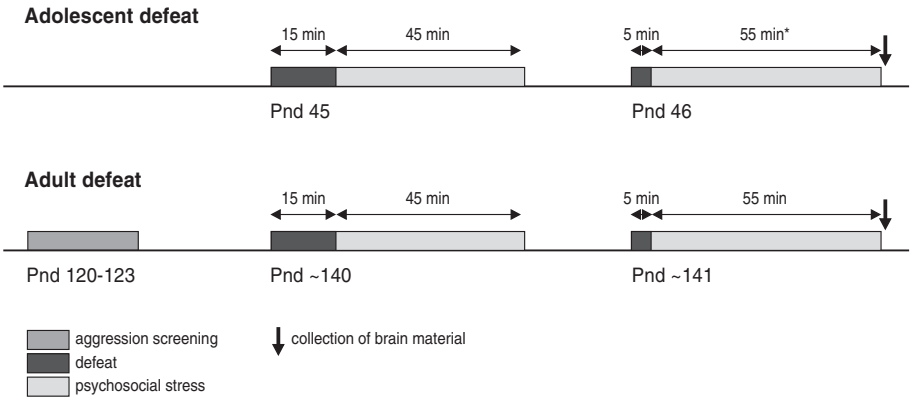

Figure 8.1 Timing of the experiment in adolescent and adult animals.

towards, nosing, investigating opponent, ano-genital sniffing, crawl over, attempted mount, social groom); 3) *Non-social exploration* (ambulation, rearing, sniffing, scanning, digging); 4) *Inactivity* (sitting, lying, immobile, freezing); 5) *Grooming* (washing, shaking, scratching).

The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals. Adult animals were divided in two groups: proactive (<15% time spent on offensive behavior) and reactive (>65% time spent on offensive behavior). All adult animals were solitary housed after aggression screening.

Social defeat

The experimental setup is illustrated in figure 8.1. Half of the adolescent and adult (proactive and reactive) animals were subjected to social defeat on two consecutive days for 1 hour. Adolescent animals were defeated at postnatal day (pnd) 45 and 46, whereas adult animals were defeat at approximately pnd 140. The resident rats were also wild-type Groningen rats and were housed in a separate room in large cages (80×55×40 cm) with a female to stimulate territorial aggression. Prior to the experimental procedure, females were removed from the resident's cage. Residents were trained to attack a naïve intruder, only residents that attacked within 2 minutes were used for the experiment. By using animals with a more or less similar readiness to attack we tried to avoid variation in attack intensity.

Experimental animals were moved to the room of the residents. Animals in the defeat groups were introduced in the cage of the resident and were attacked for 15 minutes. Thereafter animals were placed in a wire mesh cage (30×15×15 cm) in the cage of the resident for 45 minutes. In this way, animals were protected from further attacks and injury, but remained in visual, auditory and olfactory contact with the resident. This period of psychosocial stress is known to be highly stressful (Tornatzky and Miczek, 1994). On the second day of the defeats, animals were directly exposed to another aggressive resident for 5 minutes and placed in the wire mesh cage for 55 minutes. Defeats took place in the first

half of the dark phase. Brain material was collected immediately after the end of the second defeat. The control animals were directly taken from the home cage and were sacrificed at the same time as defeat animals.

Collection of brain material

Directly after the end of the second defeat, rats were anesthetized by CO_2, decapitated and the brain was removed. The prefrontal cortex, hippocampus and nucleus accumbens were rapidly dissected on ice. The tissue was immediately frozen in N_2 and stored at –80°C. The one hour time point after the start of the second defeat was chosen because previous studies indicated that the mRNAs under study are all induced within one hour after BDNF infusion into the dentate gyrus (Dagestad et al., 2006; Wibrand et al., 2006).

RNA isolation and cDNA preparation

Total RNA was isolated using the mirVana™ PARIS™ miRNA isolation kit (Ambion, AM1556) according to the manual. DNAse treatment was carried out to remove genomic DNA contamination prior to cDNA synthesis (Ambion, EN0521). The yield and quality of the RNA were determined by measuring the absorbance at 260/280 nm. Single-stranded cDNA was synthesized from 2 μg of total RNA according to the MMLV reverse transcriptase kit instructions (Ambion, AM2043).

Real-time quantitative PCR

Real-time quantitative PCR was performed on a Roche LightCycler® 480 II (Roche Applied Science) using cDNA from individual animals. cDNA corresponding to 10 ng total RNA was analyzed in 25 μl reactions using 2×TaqMan PCR mix (Applied Biosystems). PCR quantification was performed in triplicate and the relative standard curve method to determine gene expression levels was used for each animal using the Roche LightCycler® 480 Software (release 1.5.0 SP4).

Three housekeeping genes were analyzed (hrpt1, ubiquitin B and cyclophilin A) and gene expression levels for Arc, BDNF, Carp and Tieg1 were determined.

Commercially designed TaqMan®Gene expression assays were as follows (genes in parentheses): Rn00571208_g1 (Arc), Rn01484927_m1 (BDNF exon IV), Rn00572049_m1 (Carp), Rn00579697_m1 (Tieg1), Rn00690933_m1 (cyclophilin A), Rn03062801_g1 (ubiquitin B) and Rn01527840_m1 (Hrpt1). Hrpt1 expression was used as endogenous reference for the adolescent samples and adult gene expression was normalized to ubiquitin expression. The relative gene expression levels are presented as fold change based on the average group gene expression level of adolescent control and adult (reactive) control animals.

Statistics

Results are presented as mean ± SEM. Statistical analysis was performed using SPSS (version 16). Data of the adolescent social defeat were analyzed using a Student`s t-test. Adult data were analyzed using a two-way ANOVA with coping style and defeat as between subject factors. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Social defeat during adolescence leads to brain region-specific upregulation of BDNF-LTP related immediate early genes

Quantitative real-time PCR was used to determine changes in the level of mRNA expression of BDNF-LTP related genes in the prefrontal cortex, hippocampus and nucleus accumbens after adolescent social defeat. In the prefrontal cortex (figure 8.2A), the level of Arc ($p = 0.04$) and Tieg1 ($p = 0.04$) were significantly up-regulated by social defeat compared to control animals, whereas the levels of BDNF and Carp did not differ significantly between control and defeat animals. In the hippocampus (figure 8.2B), the levels

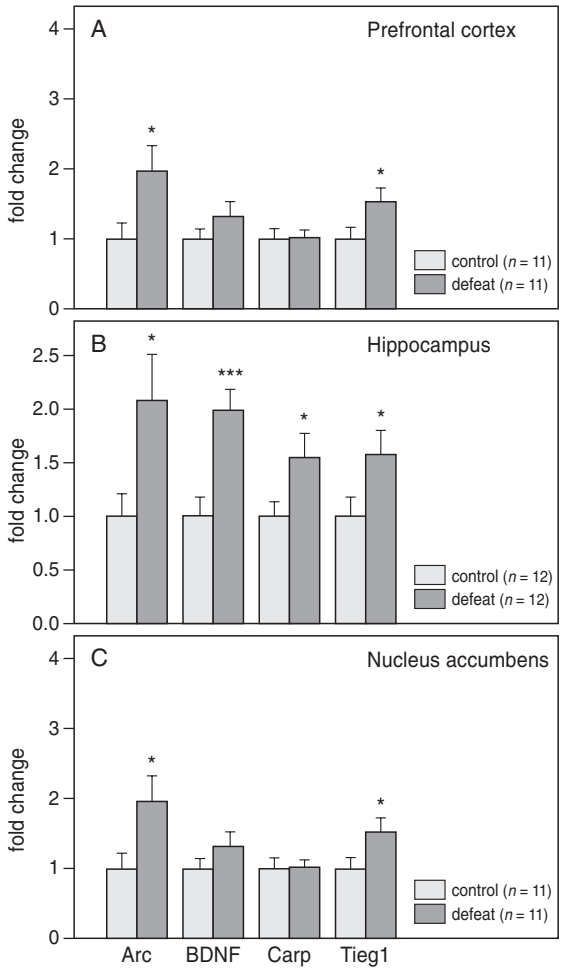

Figure 8.2 Social defeat in adolescent animals leads to brain region-specific upregulation of LTP-associated genes. Changes in mRNA expression following social defeat are expressed as fold change relative to the control group. (A) Prefrontal cortex (B) Hippocampus (C) Nucleus accumbens. Data are expressed as mean ± SEM. $*p < 0.05$, $***p < 0.001$.

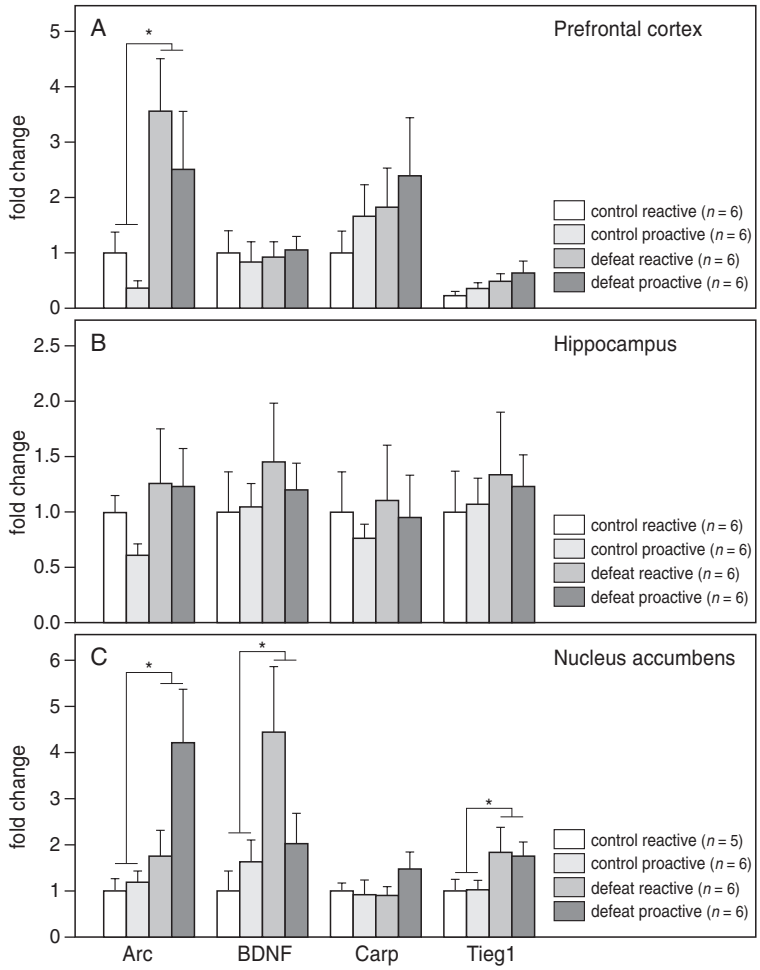

Figure 8.3 Social defeat induces brain region-specific upregulation of LTP-associated genes in adult proactive and reactive animals. Changes in mRNA expression following social defeat are expressed as fold change relative to the reactive control group (A) Prefrontal cortex (B) Hippocampus (C) Nucleus accumbens. Data are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$ from a two-way ANOVA.

Table 8.1 Behavioral profile (attack latency in seconds and percentage time spent on the six distinct behavioral categories during a 10 min resident-intruder aggression test) of the four different groups used for the study. Data are expressed as mean ± SEM. $***p < 0.001$ two-way ANOVA

	Attack latency (s)	Offense	Social explore	Social interaction	Non-social explore	Inactivity	Grooming
Control reactive	375 ± 104***	3 ± 1***	15 ± 1***	18 ± 2***	43 ± 6***	38 ± 6***	1 ± 1
Defeat reactive	545 ± 55***	3 ± 2***	17 ± 3***	19 ± 5***	57 ± 7***	22 ± 8***	1 ± 1
Control proactive	33 ± 15	83 ± 5	5 ± 3	88 ± 4	9 ± 3	1 ± 0	2 ± 1
Defeat proactive	28 ± 9	79 ± 5	4 ± 2	83 ± 5	13 ± 3	1 ± 1	3 ± 3

of Arc ($p = 0.02$), BDNF ($p < 0.001$), Carp ($p = 0.03$) and Tieg1 ($p = 0.04$) were all increased in social defeat animals. In the nucleus accumbens (figure 8.2C), the level of Arc was up-regulated 3-fold in defeat animals ($p = 0.001$), BDNF, Carp and Tieg1 levels did not differ between groups.

Social defeat during adulthood and effect of coping style

Proactive and reactive animals were divided in control and defeat groups. A justification of the composition of the four experimental groups and the behavioral profile is given in table 8.1. The data represent the time spent on five different behavioral categories and the attack latency during the 10 min offensive aggression test. There are no statistically significant differences between the various control and defeat groups.

The level of mRNA expression of BDNF-LTP related genes in adult animals is depicted in figure 8.3. The level of Arc mRNA expression was significantly elevated in the prefrontal cortex ($F_{1,19} = 9.29$, $p = 0.007$) (figure 8.3A) and nucleus accumbens ($F_{1,18} = 7.26$, $p = 0.015$) (figure 8.3C) of defeated animals, whereas Arc mRNA levels were not affected in the hippocampus (figure 8.3B). BDNF mRNA was significantly increased in the nucleus accumbens of defeat rats ($F_{1,19} = 5.00$, $p = 0.04$). A trend towards a significant interaction between the coping style of animals and defeat was seen in the level of BDNF mRNA in the nucleus accumbens ($F_{1,19} = 3.23$, $p = 0.09$) was found. Tieg1 mRNA expression levels were increased only in the nucleus accumbens ($F_{1,19} = 5.07$, $p = 0.04$). The level of Carp was not affected by social defeat in any of the examined brain regions. In the hippocampus, none of immediate early genes examined were induced in adult proactive and reactive rats, whereas all were induced following social defeat in adolescent rats.

DISCUSSION

This study shows that adolescent and adult social stress leads to brain region-specific upregulation of genes associated with BDNF-induced LTP. There is a major age-dependent effect of social defeat on gene expression in the hippocampus. Arc, BDNF, Carp and Tieg1 were all up-regulated by social stress in the hippocampus of adolescent animals, whereas in adults none of these genes were induced. Similar age dependent effects have been demonstrated by Toth and colleagues (2008). In young rats, BDNF protein levels in the hippocampus were increased after chronic mild stress, whereas decreased levels were found in the hippocampus of adult rats (Toth et al., 2008b). In addition, in young, but not adult, rats hippocampal BDNF protein induces prolonged elevations in corticosterone secretion (Taliaz et al., 2011).

In the prefrontal cortex of adolescent animals Arc and Tieg1 were up-regulated after defeat. In contrast to the hippocampus, the prefrontal cortex appears to be more affected by social defeat in adult animals. Gene expression levels of Arc are almost two-fold higher in adult defeated animals compared to adolescent defeated animals. Several studies reported an induction of Arc mRNA in the prefrontal cortex from both acute and chronic stress paradigms as well (Ons et al., 2004; Mikkelsen and Larsen, 2006; Ons et al., 2010).

The results demonstrate region-specific and age-dependent effects of social stress on immediately early genes linked to LTP and BDNF signaling. In the hippocampus, the striking age-dependent effects suggest that social defeat mobilizes a strong immediate early gene response that is absent in adult animals. This raises the possibility that hippocampal long-term synaptic plasticity is selectively engaged in social stress during adolescence. The function of the gene expression in the hippocampus is not known; it could reflect a transient adaptive response (resilience) or a step toward the experience-dependent maturation of the hippocampal response to social defeat.

It is often assumed that adolescents are more vulnerable to social stress, since the prefrontal cortex and the hippocampus are still undergoing structural reorganization during this developmental period (Kalsbeek et al., 1988; Andersen et al., 2000; Andersen, 2003). Based on the current data, we can indeed conclude that adolescent social stress is qualitatively different from adult social stress in the expression of synaptic plasticity-related genes.

We expected baseline differences in the level of genes related to BDNF-LTP in adult animals with different coping strategies. It is known that reactive coping male mice show a higher expression of several cytoskeleton gene transcripts and signal transduction genes in the hippocampus compared to proactive coping mice (Feldker et al., 2003). In addition, the intra- and infrapyramidal mossy fibers terminal fields in the hippocampus of reactive coping mice are larger (Sluyter et al., 1994). In the rat however, these observations are not supported by a difference in baseline gene expression profiles.

No difference was found in the level of gene expression between proactive and reactive coping after social defeat. However, a trend toward a differential effect of defeat on proactive and reactive coping animals was found in BDNF mRNA in the nucleus accumbens. Reactive animals show increased levels of BDNF mRNA after defeat, whereas proactive BDNF mRNA levels are comparable to levels of control animals.

The differential BDNF mRNA expression after defeat in reactive and proactive animals might be associated with resilience to social defeat. Krishnan and colleagues showed that BDNF mRNA levels in the nucleus accumbens of mice subjected to social defeat are equal to control animals. However, in a group of susceptible mice BDNF protein levels are increased, whereas BDNF levels in unsusceptible mice are unaffected (Krishnan et al., 2007). Manipulation of BDNF gene expression levels in the mesolimbic dopamine pathway by local deletion of the BDNF gene reduces the long-term neural and behavioral response to social defeat stress similar to effects produced by antidepressant treatment (Berton et al., 2006).

Arc is a key effector protein for BDNF-induced LTP, but Arc is multifunctional protein required for other forms of synaptic plasticity such as long-term depression and homeostatic scaling (Rial Verde et al., 2006; Shepherd et al., 2006; Bramham, 2008; Waung et al., 2008) as well. Homeostatic plasticity may compensate LTP and LTD by scaling neuronal output without changing the relative strength of individual synapses (Shepherd et al., 2006). Synaptic plasticity in the developing visual cortex is an example of this homeostatic plasticity. Arc appears to be required for the experience-dependent processes that normally establish and modify synaptic connections in the visual cortex (McCurry et al.,

2010). Arc induction after social defeat might induce a similar process of homeostatic plasticity.

It is unknown what mechanism determines whether Arc is selectively engaged in homeostatic plasticity, LTP or LTD. Social defeat has not only been shown to reduce LTP in rats, but at the same time enhances long-term depression (LTD) 7-9 months after repeated defeat experience (Kole et al., 2004; Artola et al., 2006). Therefore, the increased level of Arc found after social defeat in the current study might be functionally involved in the process of homeostatic plasticity.

One confounding factor may be that the current social defeat procedure includes individual housing after social defeat. Social isolation appears to be an important factor in the long term effects of defeat since social housing has been shown to reduce the impact of social defeat (Ruis et al., 1999; De Jong et al., 2005). Solitary housing in itself affects LTP and LTD, LTP is higher in animals that are housed in an enriched environment compared to individually housed animals (Artola et al., 2006).

Other possible confounding factors are false-negative results and the temporal dynamics of gene expression after social defeat. The fact that all genes were up-regulated in the hippocampus of adolescent rats makes it unlikely that there are false-negatives in the gene expression levels of other brain areas of adolescent and adult animals.

The temporal dynamics of mRNA expression following BDNF-LTP and high frequency stimulation (HFS) LTP has been studied by Wibrand and colleagues using in situ hybridization. They showed that the kinetics of mRNA was rapid (40 minutes following post-HFS) and sustained (3 hours post BDNF) in the dentate gyrus granule cells (Wibrand et al., 2006). Based on this time course of gene expression it is unlikely that the chosen time point resulted in false-negative effects of social defeat.

We did not dissociate between sub-regions of the hippocampus, prefrontal cortex and nucleus accumbens. However, there may be a difference in gene expression levels in different regions of the hippocampus. For example, Grønli and colleagues showed that chronic mild stress inhibits BDNF protein expression in the dentate gyrus, but not in the hippocampus proper (Grønli et al., 2006) and immobilization stress in rats is associated with greater impairments in BDNF mRNA expression in the dentate gyrus compared to the cornus ammonis (CA) region (Smith et al., 1995). Similarly, the prefrontal cortex and nucleus accumbens can be divided in different regions and gene expression levels induced by stress can differ between the different regions (Ons et al., 2004). Whether subregional differences in gene expression in response to social defeat stress exist needs to be determined in future experiments using in situ hybridization.

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CHAPTER 9

General discussion

INTRODUCTION

Adolescence is generally considered as a developmental period during which adverse social experience may have lasting consequences in terms of the development of psychopathology. Most research in this area focuses on depression as a consequence of early life stress (Edwards et al., 2003; Heim and Binder, 2012). Psychiatry considers depression as an internalizing disorder as opposed to externalizing disorders such as antisocial personality disorders and heightened aggression. While internalizing disorders are directed inward and are an indication of the emotional and psychological state of an individual, externalizing behaviors are displayed outwardly and are directed towards the physical environment (Liu et al., 2011).

Interestingly, while women show significantly higher prevalence rates of internalizing disorders (Hankin et al., 1998; Kendler et al., 2002; Wade et al., 2002; Wittchen and Jacobi, 2005; Bouma et al., 2008), men show significantly higher rates of externalizing disorders (Kessler et al., 1993; Kessler et al., 1994; Nolen-Hoeksema, 2012). The relationship between early life stress and the development of externalizing disorders has hardly been studied so far. In this thesis I focused on the consequences of adolescent social stress on aggression, violence, impulsivity and the underlying neurobiology in males.

Regulated aggressive behavior can transform into an externalizing disorder expressed as the display of uncontrolled violent forms of behavior. Violence can be defined as escalated aggressive behavior that is expressed out of context and out of inhibitory control, and does not to serve an adaptive function in social communication (De Boer et al., 2009a; Natarajan and Caramaschi, 2010).

Violence is a major contributor to public health problems worldwide. The World report on violence and health launched by the World Health Organization (WHO) in 2002 estimated that each year, approximately 1.6 million people die as a direct result of injuries resulting from interpersonal violence (Krug et al., 2002).

There is a paucity of psychological, psychiatric and biological studies addressing the factors that contribute to the transition of aggressive behavior into violent behavior. It is unclear which intrinsic and environmental modulating factors are involved in this transition. However, in view of the available literature, it is likely that a lack of impulse control is contributing to the escalation of aggressive behavior.

Both aggression and impulsivity have been shown to be under direct control of the prefrontal cortex in humans (Davidson et al., 2000; Blair, 2004; Siever, 2008). The prefrontal cortex is still developing during adolescence (Kalsbeek et al., 1988; Giedd et al., 1999; Andersen et al., 2000; Spear, 2000; Andersen, 2003; Tseng and O'Donnell, 2007) and I hypothesized that disturbance of this development by adverse social experience might lead to a long-lasting increase in the level of aggressive and impulsive behavior.

These long lasting changes in behavior after adolescent social stress might be mediated by changes in synaptic plasticity in the prefrontal cortex. Indeed, changes in synaptic plasticity have been associated with major depression and defective synaptic plasticity in the prefrontal cortex is linked externalizing disorders (Dickstein et al., 2006; Brennan and Arnsten, 2008).

Finally, it is well known that personality characteristics are an important factor in vulnerability to disease. Therefore, I used two widely used models of animal personality to study the individual differences in the susceptibility to social stress during adolescence in males and used synaptic plasticity markers, adult levels of offensive aggression and impulsivity as read-outs.

SUMMARY OF THE RESULTS

The main aim of the work described in this thesis was to study the consequences of adolescent social stress on adult aggression and impulsivity, with special emphasis on individual differences in susceptibility. Furthermore, I tested the hypothesis that the behavioral outcome of social defeat experienced during adolescence is modulated by synaptic plasticity in the prefrontal cortex.

In the **chapter 2** I described the relationship between coping styles and behavioral flexibility/impulsivity. Aggressive behavior was negatively correlated with behavioral flexibility. I argued that the consistency of correlations between behaviors across contexts should be explained by the individual variation in common underlying proximate mechanisms.
In **chapter 3** and **4** I characterized animals of the wild-type Groningen (WTG) rat strain and rats of the Roman selection lines in terms of aggression and behavioral flexibility / impulsivity based on the assumption that both behaviors reflect prefrontal cortex functioning. I showed that offensive aggression is indeed correlated with behavioral flexibility, as measured in an unpredictable operant conditioning paradigm for food reinforcement (variable interval 15, VI-15) in WTG rats. Behavioral inhibition was not different between high and low aggressive animals, but violent animals show low levels of behavioral inhibition.

In rats of the Roman selection lines we were not able to show the correlation between offensive aggression and behavioral flexibility (**chapter 4**). Although proactive coping RHA rats are characterized by high levels of active avoidance in a shuttle-box task and low levels of behavioral flexibility, this was not related to their level of offensive aggression.

The question whether individual differences in impulsivity are accompanied by differences in the cardiovascular response of animals was the topic of **chapter 5**.

I hypothesized that individual differences in behavioral flexibility are accompanied by differences in the cardiovascular response of animals. The blood pressure and heart rate response were qualitatively related to behavioral performance on the different reinforcement schedules. However, the quantitative behavioral response was not related to the coping style of animals.
In **chapter 6** and **7**, I studied the consequences of social defeat during adolescence on adult offensive aggression and impulsivity. Furthermore, I studied the stability of behavioral flexibility between adolescence and adulthood in WTG rats (**chapter 6**). Although the mean level of behavioral flexibility did not change between adolescence and adulthood, I did not find a correlation between adolescent and adult VI-15 performance.

Social defeat during adolescence led to a disturbed relationship between aggression and lever press performance on a VI-15 task in WTG rats. In rats of the Roman selection

lines, RHA rats became more efficient on the VI-15 schedule, whereas the performance in RLA rats was not affected by adolescent social defeat. The hypothesis that social defeat during adolescence would lead to an increase in aggressive and less behavioral flexibility was not confirmed.

I compared the consequences of social defeat on the expression of BDNF-regulated immediate early genes in adolescence and adulthood in **chapter 8**. In general, I showed that social defeat leads to an up-regulation of these genes. A differential effect of social defeat in adolescent and adult animals was found in the hippocampus. We found an up-regulation of BDNF-regulated immediate early genes in the hippocampus of adolescent animals, whereas this increase was not found in adult animals.

Taken together, to show that adolescent social stress can lead to externalizing disorders in males, I first showed that aggression and behavioral flexibility are two related constructs in adulthood. Thereafter, I demonstrated that adolescent social defeat leads to a disturbance of the relationship between aggression and behavioral flexibility. The response pattern of proactive and reactive animals seems to be different. Furthermore, adolescent and adult animals show a differential neuronal response to social defeat stress. Further research is needed to determine which phenotype and environmental conditions determine whether exposure to adolescent social stress leads to externalizing or internalizing disorders.

PERSONALITY IN ANIMALS

One of the core hypotheses of this thesis is that individuals may differ strongly in susceptibility to the long term consequences of adolescent social defeat. In addition, social experiences during adolescence may be an important factor shaping the adult phenotype. Individual variation in behavior is often summarized using the term personality. This term is clearly derived from human research but requires some further discussion and explanation in the context of the current thesis.

Nowadays, the 'big five' model for human personality is often adopted. The five factors (openness, conscientiousness, extraversion, agreeableness, neuroticism) for human personality originated from cluster analysis of behavioral traits (Goldberg, 1990). This cluster analysis is not theory driven, but merely a data-driven exploration. Proximate mechanisms explaining the clustering of behaviors are largely lacking. From the point of view of behavioral neuroscience, it is reasonable to suggest that behaviors are correlated because they share the same neurobiological, neuroendocrine and/or genetic mechanisms (Bell, 2007a; Bell et al., 2007).

The information obtained in animal models may contribute to a neurobiologically underpinned conceptual framework of personality in general. The two-tier model of coping styles is frequently used as a simplified model of personality in animals (Steimer et al., 1997; Koolhaas et al., 2010). We proposed that the coping style axis reflects how an animal responds to a challenge (qualitative dimension) and that the emotional reactivity axis reflects how strongly it responds (quantitative dimension).

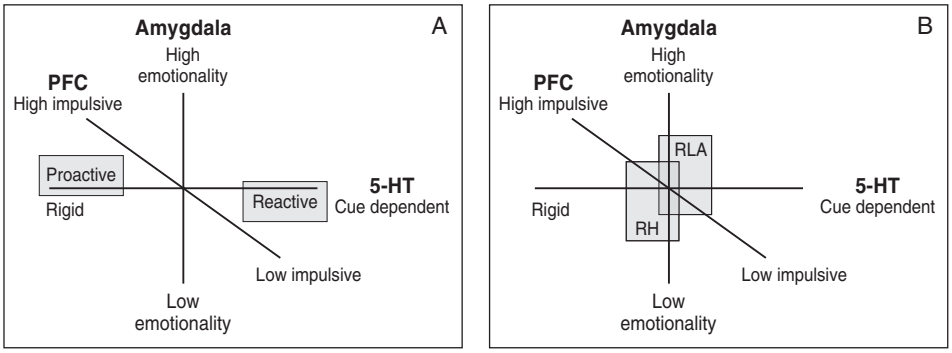

Figure 9.1 Coping style model (A) The position of rats of the wild-type Groningen strain in the proposed coping style model. (B) The position of rats of the Roman selection lines in the proposed coping style model. Abbreviations: 5-HT, serotonin; PFC, prefrontal cortex.

Based on the experiments performed for this thesis and on the literature, I propose an adapted model in which the axes are explained in terms of the underlying neurobiological substrates (figure 9.1). The hypothesized position of proactive and reactive wild-type Groningen rats (figure 9.1A) and rats of the Roman selection lines (figure 9.1B) in this model is also indicated.

The first axis of the proposed model represents flexibility of behavior. Flexible behavior is defined as being open to external cues. It is likely that performance on the variable-interval schedule for food reinforcement reflects flexibility of behavior. Low aggressive individuals appear to pay attention to the drop of the food pellet into the food receptacle, whereas high aggressive animals keep pressing the lever at a high rate without paying attention to the delivery of food pellets on the VI-15 schedule of reinforcement.

The display of flexible, cue dependent behavior is associated with a high level of serotonin (Homberg, 2012). On the other hand, proactive, high aggressive individuals show rigid behavior (Benus et al., 1990) which is associated with low levels of serotonin (Caramaschi et al., 2007; Cervantes and Delville, 2007; Koolhaas et al., 2007; Caramaschi et al., 2008; Cervantes and Delville, 2009). Taken together, it is likely that differences in behavior related to the flexibility axis of the proposed coping style model are mediated by the amount of the neurotransmitter serotonin.

The flexibility axis shows a strong similarity with the openness dimension of human personality. Indeed, openness to experience is also in humans negatively correlated with cerebral serotonin transporter (5-HTT) binding (Kalbitzer et al., 2009). Children homozygous for the S allele of the promoter polymorphism of the serotonin transporter gene (5-HTTLPR), which is associated with reduced 5-HTT gene transcription, have lower scores on the openness dimension (Harro et al., 2009).

The second axis of the proposed model represents emotionality, which is mediated by amygdala functioning. Rats of the Roman selection lines have been shown to differ in the involvement of the amygdala in their behavior. Experimental evidence shows that the divergent behavioral response to anxiogenic stimuli is accompanied by different activation

of the amygdala (Wiersma et al., 1998; Meyza et al., 2009). Roman low avoidance (RLA) rats are characterized by high emotional reactivity, whereas Roman high avoidance (RHA) rats respond with a low emotional response to challenges (Steimer and Driscoll, 2003). At the same time, RHA rats are characterized by low behavioral flexibility (**chapter 4**) and high levels of impulsive behavior (Zeier et al., 1978; Moreno et al., 2010). It is likely that impulsivity should be considered as an independent dimension in the coping style model, since it is not related to levels of offensive aggressive behavior and VI-15 performance (**chapter 3**). A more general discussion about the difference between behavioral flexibility and impulsivity will follow in the next section.

Lesion studies show that impulsive behavior is mediated by prefrontal cortex functioning (Mobini et al., 2002; Winstanley et al., 2006a; Dalley et al., 2008). The prefrontal cortex plays an important role in the inhibitory control over behavior. Inactivating the orbital frontal cortex leads to the preference of a small immediate reward instead of a large delayed reward (Mobini et al., 2002). Unfortunately, studies on the role of the prefrontal cortex in individual differences in rats of the Roman selection lines are lacking.

IMPULSIVITY

Impulsivity is generally defined as 'action without foresight'. Due to this very broad definition of impulsivity, a large number of behavioral acts can be categorized under impulsivity. Moreover, this definition cannot be used for animal studies because foresight cannot be determined directly. In the experiments described in this thesis I used two different operant conditioning paradigms for food reinforcement (variable interval 15 and differential-reinforcement-of-low-rate (DRL) responding) as a proxy of impulsive behavior. Although the behavioral parameters in the two schedules have some overlapping aspects, they are largely dissociable in other terms.

On a variable interval paradigm, the number of responses varies tremendously between animals and is correlated with offensive aggressive behavior (**chapter 3**). However, the outcome in terms of rewards is similar. Some animals do not inhibit their behavior and press a lever a lot in this paradigm whereas others pay more attention to the environment (dropping of a food pellet) and press the lever less. Therefore, the results of this test can be seen as different strategies with an equal benefit.

The results on the VI-15 schedule of reinforcement may be better explained in terms of flexibility or cue dependency rather than impulsivity. Reactive coping animals are more flexible on this reinforcement schedule. They press the lever less, since they are paying more attention to the drop of a food pellet in the food receptacle. Once they notice a food pellet has been delivered, they stop pressing the lever and first consume the pellet. Proactive coping individuals, on the other hand, show more routine-like behavior. These animals pay less attention to the drop of the food pellet and keep on pressing at a high rate until there is a pile of food pellets. An interpretation of the VI-15 results in terms of flexibility is consistent with a range of other experiments (Benus et al., 1990; Bolhuis et al., 2004) showing that cue dependency is one of the main differences between proactive

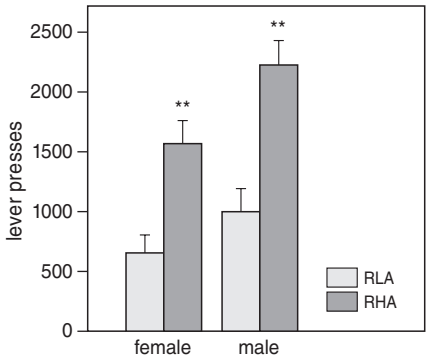

Figure 9.2 Performance on a VI-15 schedule in RLA (n = 6) and RHA (n = 6) female and male rats. The data are expressed as the total number of presses ± SEM during a one hour VI-15 session. RHA rats performed a significantly higher number of lever presses compared to RLA rats both in females (p=0.003) and in males ($p < 0.002$). **$p < 0.01$.

and reactive coping. This also fits to the idea that reactive animals are doing better under changing environmental conditions. Proactive coping animals will do better in stable conditions, because they make faster decisions and show rigid behavior.

On the DRL scheme, the ability to wait for a reward can be determined and behavioral inhibition as measured by bursting behavior. Animals with different coping styles respond similarly with respect to distribution of responses in the pause interval, which measures the ability to wait for reward. Violent animals, on the other hand, show higher levels of impulsive behavior on the DRL scheme compared to 'normal' and low aggressive animals. This indicates that a lack of impulse control may be an important aspect of the pathology of aggression and externalizing disorders.

Inevitably, the question arises if the relationship between aggression and impulsivity is gender specific. Men are generally considered to be more impulsive than women (Esteban and Tabernero, 2011) and in an animal model for ADHD, a higher level of impulsive behavior in males has also been found (Berger and Sagvolden, 1998). Performance on a differential-reinforcement-of-low-rate (DRL) responding schedule in females and males of the Roman selection lines has been compared by others. They showed that RLA females were less impulsive (more efficient) than RHA females, whereas males of the two strains tended towards middle scores (Zeier et al., 1978).

We tested the level of behavioral flexibility using a variable interval schedule in females of the Roman selection lines. The results of this experiment are shown in figure 9.2. The difference in performance in male RLA and RHA rats (**chapter 4**) is also present in females. Unfortunately, a direct answer to the question of gender differences in impulsivity cannot be given because a direct comparison of the quantitative level of performance is not possible, since females and males were tested in two separate experiments.

It is impossible to relate offensive aggression with impulsive behavior in female wild-type Groningen rats, since female rats do not show offensive aggression in a resident-intruder paradigm. However, the current results of the Roman selection lines are in line

with the general idea that females of genetic selection lines of coping style differ in the same direction as the males (Aguilar et al., 2003).

The discussion above illustrates that the same term (i.e. impulsivity) is used for different behavioral physiological processes and confusion may arise. For instance, the term behavioral inhibition is used for distinct inhibitory processes. In at least three different paradigms the term behavioral inhibition is used and they are all clearly different from each other. The inhibition of the response to opt for an immediate small reward to obtain a delayed larger reward ('delayed gratification') is different from the inhibition of a motor response ('stopping') or inhibition in a reversal learning paradigm (Humby and Wilkinson, 2011).

Another problem is that the same term is used in different research fields. For example, the use of flexibility of behavior is both used in ecology and in behavioral biology. In the field of behavioral biology, adaptations of behavior on the short term are called flexible behavior. Behavioral biologists for example test flexibility in maze tasks (Benus et al., 1990) and unpredictable operant conditioning tasks. On the other hand, ecologists use the term plasticity to indicate that the expression of behavioral traits is not fixed within genotypes or individuals (Dingemanse et al., 2007). This form of flexibility obviously does not reflect impulsive behavior but is more related to the concept of phenotypic plasticity (Pigliucci, 2005; Auld et al., 2010).

The previous examples show that attention should be paid to the definition of impulsivity and flexibility of behavior. The definitions should preferably be based on the mechanisms supporting behavior, if behaviors are similar and mediated by the same mechanism, the same term can and should be used.

To test if the same mechanisms are involved, temporary inactivation of the expected involved neuronal substrate and testing performance in different behavioral paradigms will give an indication whether this really is the case. It is important to use such an approach in order to avoid the use of one term for different processes.

NEUROBIOLOGICAL SUBSTRATES

The common denominator of the experiments in this thesis is the prefrontal cortex. This brain structure matures during adolescence (Kalsbeek et al., 1988; Giedd et al., 1999; Andersen et al., 2000; Spear, 2000; Andersen, 2003; Tseng and O'Donnell, 2007) and has been associated with both aggressive behavior (Blair, 2004; Siever, 2008) and various aspects of impulsive behavior (Mobini et al., 2002; Winstanley et al., 2006a; Dalley et al., 2008). Several studies in human subjects show that gray matter volume of the prefrontal cortex is related to both aggressive and impulsive behavior (Antonucci et al., 2006; Gansler et al., 2011). Studies performed by Delville and colleagues in hamsters also show that aggression and impulsive behavior are two related behaviors and they speculate that this is dependent on the prefrontal cortex (Cervantes and Delville, 2007).

In the experiments described in this thesis, I show that offensive aggressive behavior is correlated to performance in an unpredictable operant conditioning paradigm (**chapter 3**).

Whether this correlation is causal and caused by differences in prefrontal cortex functioning cannot be concluded from these experiments.

Unfortunately, due to a lack of time I have not been able to test this causal relationship. The long term consequences of stress during adolescence might be mediated by the prefrontal cortex, due to disturbance of ongoing development of this brain structure in adolescence (Kalsbeek et al., 1988; Giedd et al., 1999; Andersen et al., 2000; Spear, 2000; Andersen, 2003; Tseng and O'Donnell, 2007). Nevertheless, one may wonder whether the prefrontal cortex is the sole or key structure underlying the consequences of adolescent social stress or if other brain structures are involved as well.

During adolescence, emotional experiences become more intense and this change in emotional processes is accompanied by an increase in volume of the amygdala until early adulthood (Giedd et al., 1996). The size and activity of the amygdala are increased in major depression (reviewed in: (Drevets, 2003)). The amygdala is the brain structure that is known to be involved in emotional processing (LeDoux, 2000; Sergerie et al., 2008).

As mentioned above and in **chapter 2** the amygdala can be seen as the underlying neurobiological substrate of the emotional reactivity axis of coping style model. The display of aggression is associated with heightened neuronal activation in the central amygdala (Veenema and Neumann, 2007) as well. Lesion of the amygdala reduces aggressive behavior in rats with previous victory experience (Vochteloo and Koolhaas, 1987). Similar lesions of the basolateral amygdala promote impulsive behavior (Winstanley et al., 2004a; Cardinal, 2006; Zeeb and Winstanley, 2011).

Behaviors are not induced by activation of just one brain structure, but rather by activation of neural networks (Newman, 1999). Besides the prefrontal cortex, the amygdala is a node in the network(s) involved in aggressive behavior (Newman, 1999; Goodson, 2005) and impulsivity (Eagle and Baunez, 2010).

Social defeat also induces changes in the mesocorticolimbic dopaminergic system, which might mediate long term consequences on reward related behavior. Social defeat leads to increased mu-opioid receptor mRNA expression in the ventral tegmental area (VTA), which modulates dopamine transmission in this area and causes a sensitized response to psychostimulants drugs (Nikulina et al., 2004; Covington et al., 2005; Nikulina et al., 2008).

The results of **chapter 8** show that social defeat stress not only leads to an increase in the expression of BDNF-regulated immediate early genes in the prefrontal cortex, but also leads to a heightened expression level of these genes in the hippocampus and nucleus accumbens. These data support the view that social defeat leads to neuronal network changes, rather than to a change in one brain structure.

BRAIN DEVELOPMENT

The postnatal brain still develops and does not fully mature until late adolescence. Several basic processes such as neurogenesis, neuronal migration, synaptogenesis, pruning, etc. underlying brain development occur during this long developmental trajectory (Lewis and

Levitt, 2002; Jaaro-Peled et al., 2009; Koenig et al., 2011) and stress during this period may lead to permanent neurobiological and behavioral changes (Maccari and Morley-Fletcher, 2007; Weinstock, 2007).

Contemporary views in behavioral neuroscience emphasize the view that the brain is a highly dynamic organ. Adolescence is characterized by changes in brain structure and volume. In general, cortical grey matter volume increases during childhood, reaches peak levels around the start of adolescence and then gradually decline (Giedd et al., 1999). White matter volume shows a more linear increase throughout childhood and adolescence (Pfefferbaum et al., 1994).

In contrast, during aging, significant decreases in brain volume are taking place. Parts of the frontal cortex are shrinking with aging and the volume of the amygdala decreases around the age of 60 as well (Giedd et al., 1996; Tisserand et al., 2002). Excess glucocorticoids in aged rats lead to hippocampal dendritic atrophy and inhibit neurogenesis (Landfield et al., 2007; Lupien et al., 2009). Taken together, periods in which the brain is developing are periods where environmental factors may have more lasting consequences on the brain and behavior and this is not limited to adolescence.

Furthermore, several issues about the relationship between adolescent social stress and adult psychopathology are still unclear. For example, the effect of negative life experiences on brain and behavior might not be visible immediately, but can be expressed later on. The steep increase in incidence rates for psychiatric disorders (Bernstein et al., 1996; Hankin et al., 1998) during adolescence might be either the long-term consequence of negative experiences before adolescence or this increase is the immediate result of a negative impact of stress experienced during adolescence.

Another factor that might contribute to the rise in the incidence rate of psychiatric disorders is a change in report rates by parents around adolescence. Since individuals are behaviorally changing tremendously around adolescence, parents may become more aware of deviant behavior and therefore report rates for psychiatric disorders may increase around this period in life.

These questions justify the use of an animal model to study the consequences of social stress during adolescence on adult behavior. By using an animal model we could avoid the possibility that the effects originated pre- or (early) postnatally or are the result of a change in report rates of parents and we could isolate the effects of adolescent social defeat.

ADOLESCENCE: A SENSITIVE PERIOD?

Social stress during adolescence is often claimed to have major impact on the development of psychiatric diseases. However, it is unclear whether the behavioral and neurobiological consequences of stress experienced during adolescence are age specific or whether they are similar to the adult consequences of stress. Surprisingly, studies directly comparing the consequences of stress during adolescence and adulthood are scarce.

In a comparison of the impact of restraint stress on cFos expression in adolescent and adult animals, Sui and co-workers showed a higher cFos expression in the hippocampus of

adult animals compared to adolescent animals (Sui et al., 2010). Others found similar age specific effects with respect to cFos expression in the amygdala after restraint stress in adolescent and adult animals (Kellogg et al., 1998).

Some studies have been performed at a mechanistic level. These studies involve the difference between adolescence and adulthood in the hypothalamic-pituitary-adrenal (HPA) axis response to stress. This neuroendocrine axis that mediates the stress response, responds in a different fashion to stress in early adolescence and in adulthood. Pre-pubertal animals have a prolonged ACTH (adrenocorticotrophic hormone) and corticosterone response compared to adults and more cFos expression in the paraventricular nucleus (PVN) of the hypothalamus after an acute stressor (Romeo et al., 2006; Romeo and McEwen, 2006).

Environmental factors that interfere with the hormonal changes that occur during adolescence are likely to contribute to the adult vulnerability for psychopathologies. Several studies indicate for example that the HPA-axis response in girls is different from those in boys and more influenced by genetic factors and prior environmental exposures (Reviewed in: (Oldehinkel and Bouma, 2011). The time course of development of the hypothalamic-pituitary-adrenal (HPA) axis is different between females and males (Pignatelli et al., 2006) and may therefore account for differences in stress induced behavioral and physiological effects in females and males. Indeed, the prevalence of depression changes between pre-puberty and puberty from equal to a twofold higher prevalence in females compared to males (Hayward and Sanborn, 2002; Angold and Costello, 2006). At the same time, interpersonal stressors are especially highly prevalent for girls in adolescence.

At the behavioral level, there are a few studies indicating that stress experienced during adolescence or adulthood has differential effects. Bingham compared the effects of stress experienced early in adolescence, in the middle of the adolescent period and during adulthood. This study showed that social defeat during adolescence results in opposite effects on burying behavior compared to adult animals. Rats defeated during early adolescence show more burying behavior compared to age-matched controls (Bingham et al., 2011). Unfortunately, only short-term consequences of stress were investigated and relatively young adult animals (~pnd 65) were used. At this age the animals are behaviorally still relatively immature.

Although a differential effect of stress during adolescence and adulthood on impulsive behavior has not been compared so far, a recent study indicates that there might be clear effects. Treatment of adolescent animals with corticosterone increases impulsive choice while simultaneously decreasing impulsive action (Torregrossa et al., 2012). In my studies on the consequences of social defeat on impulsive behavior I did not find a clear effect of adolescent social defeat on adult VI-15 performance (**chapter 6** and **7**) and could not confirm that adolescent impulsivity levels are higher than adult levels of impulsive behavior (**chapter 6**) (Kelly and Deadwyler, 2003; Laviola et al., 2003; Andrzejewski et al., 2011).

Based on the experiments performed in **chapter 8** in which I compared the consequences of social defeat stress on synaptic plasticity in adolescent and adult animals and taking into account the literature mentioned above, one might conclude that stress

experienced during adolescence has different behavioral and neurobiological consequences than stress during adulthood. It is unclear whether this means that adult individuals experiencing stress are better capable to deal with the consequences of stress, or the contrary, that they are more vulnerable.

VULNERABILITY OR RESILIENCE?

Although adolescence is a period of major social and psychological changes, most adolescents are developing well. Even though a substantial number of adolescents are exposed to chronic stress in life, the majority does not develop serious psychological disorders (Luthar, 1991; Masten, 2001). Hence, the question rises whether adolescence is a particularly vulnerable period or a period in which they develop resilience. Resilience refers to the idea that an individual is better capable to cope with stress.

Adolescence is a period characterized by rapid environmental changes. A changing environment requires adaptation in order to achieve optimal survival. Individuals have a certain adaptive capacity, which results in a certain degree of resilience to change (figure 9.3A). However, adaptations might appear to be insufficient or even maladaptive when the prediction about the environment turns out to be incorrect. In stress research this is termed allostatic load: the cost of wear and tear on the body produced by repeated activation of biological stress response systems (McEwen, 1998; Karatsoreos and McEwen, 2011).

The cumulative stress or double hit hypothesis of disease (figure 9.3B) is based on the idea of allostatic load. This hypothesis assumes that when individuals are exposed to stress early in life, this adds to their allostatic load and they will be more vulnerable to stress later on. The effects of stressors are considered to be cumulative and build up to a higher allostatic load which in turn increases the likelihood of developing disease (Nederhof and Schmidt, 2011).

An alternative view is given by the mismatch hypothesis of psychiatric disease (figure 9.3C). This hypothesis proposes that adaptations to an adverse environment may be beneficial when the environment remains adverse. Animals may learn to cope with adverse environmental conditions and are therefore be resilient to the consequences of stress. The risk for the development of disease depends in this view largely on the match between the early environment and the conditions at a later stage in life (Nederhof and Schmidt, 2011; Schmidt, 2011).

One of the applications of the mismatch hypothesis of disease is shown by Champagne and colleagues. They showed that differences in maternal care modulate optimal cognitive functioning in adulthood. Offspring of low licking and grooming mothers do better under high stress conditions, whereas high licking and grooming offspring show enhanced learning under contexts of low stress (Champagne et al., 2008).

When applied to adolescence, the mismatch hypothesis implies that adolescence might also be seen as a period of opportunity. This is supported by the observation that the effects of prenatal stress can be reversed by an enriched environment during adolescence. Prenatally stressed animals show increased anxiety related behaviors, increased HPA axis

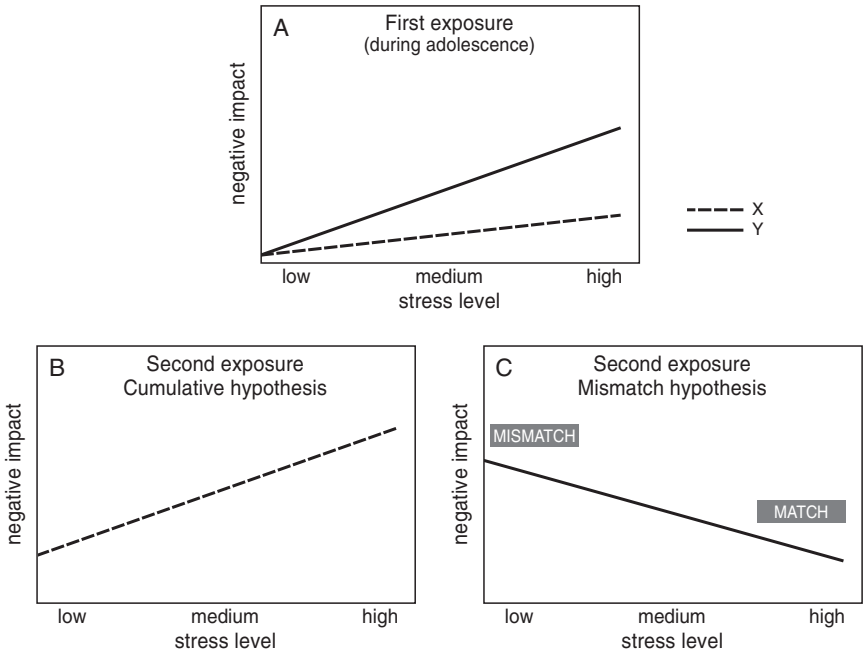

Figure 9.1 (A) Hypothesis on the impact of adolescent stress and the consequences of a second exposure to stress. (B) Cumulative stress hypothesis. I hypothesize that reactive animals (phenotype X) respond according to this model (C) Mismatch hypothesis. I expect that the behavioral response of proactive animals (phenotype Y) follows this curve. The response to the second exposure to stress is depending on the existing environmental conditions.

reactivity and reduced play behavior. These effects are absent in animals raised in an enriched environment (Morley-Fletcher et al., 2003; Laviola et al., 2004). Similar results are obtained in animals stressed due to suboptimal maternal care growing up in an enriched environment (Francis et al., 2002; Bredy et al., 2003; Bredy et al., 2004). The studies mentioned above show that there is some experimental evidence for both of the hypotheses (Bilbo et al., 2008; Champagne et al., 2008; Veenema et al., 2008; Uchida et al., 2010).

The results of the experiments described in **chapter 7** of thesis indicate that the behavioral profile of reactive coping individuals does not change after adolescent social stress. On the other hand, the adult behavioral profile of proactive animals is changed after defeat stress experienced during adolescence. This leads to the testable hypothesis that the cumulative stress theory might be applicable to reactive animals and the mismatch hypothesis might be valid for proactive individuals.

True experimental evidence for the applicability of the hypotheses of disease could be obtained by subjecting individuals defeated during adolescence to a second social defeat in adulthood and analyzing the development of psychopathology (Buwalda et al., 2013). This would be a valuable approach to come to a more general model of the development of disease.

In conclusion, it is not the question whether adolescence is a period of vulnerability or resilience. Dependent on environmental condition, phenotype and adolescent social stress may both contribute to vulnerability and resilience. Stressful experiences in adolescence might predispose some individuals to disease, whereas others will do better under stressful conditions in adulthood.

SYNAPTIC PLASTICITY

Research of the last decades shows that the processes of synaptic plasticity are at the core of virtually all behavioral adaptation. Synaptic plasticity is not only a core process of learning and memory, it is more generally the process by which the environment shapes the brain throughout life.

Perturbations in synaptic plasticity have been connected to major depression and are thought to play an important role in pathogenesis of diseases across the central nervous system. For example, defective synaptic plasticity in the prefrontal cortex is linked to attention-deficit and impulse-control disorders (Dickstein et al., 2006; Brennan and Arnsten, 2008).

In view of its central role as a major regulator of synaptic transmission and plasticity at adult excitatory synapses (Bramham and Messaoudi, 2005), I focused on the neurotrophic factor BDNF (Brain-derived neurotrophic factor) as a marker for synaptic plasticity. It regulates experience dependent changes in synaptic connectivity, is implicated in stress-induced or reactive depression and the therapeutic effects of antidepressants (Castren and Rantamaki, 2010). It is involved in both the induction and consolidation of long-term potentiation (LTP).

In **chapter 8** I showed that both adolescent and adult social stress lead to brain region-specific up-regulation of genes associated with BDNF-induced LTP. The pattern of gene expression levels after social defeat is different in the prefrontal cortex, hippocampus and nucleus accumbens. In addition, I showed that the effects of social defeat on the gene expression levels are age-dependent. Based on these data I concluded that adolescent social stress is qualitatively different from adult social stress in the expression of synaptic plasticity-related genes.

However, the functional implication of this response and the specificity for the BDNF pathway is not clear. In general, it is unknown how synaptic reorganization affects neural activity and cognitive function. Furthermore, we do not know whether increased expression of Arc and BDNF mRNA causes an increase in the number of synapses or strengthening of existing synapses.

Arc has been shown to be involved in both the induction and consolidation of long-term potentiation (LTP). However, recent evidence suggests that there is a competition between “anabolic” (mature-BDNF – TrkB signaling) and “catabolic” (pro-BNDF – p75 signaling) effects on neuronal plasticity. This implicates that there is bi-directionality of neuronal plasticity. Increases in neurite arborization, synaptogenesis and neurogenesis are balanced by programmed neuronal death, neurite retraction and synaptic pruning (Castren and Rantamaki, 2010).

Arc is a multifunctional protein required for other forms of synaptic plasticity such as mGluR-dependent long-term depression (LTD) and homeostatic scaling as well (Rial Verde et al., 2006; Shepherd et al., 2006; Bramham et al., 2008; Waung et al., 2008). These effects are mediated through interaction of Arc with components involved in endocytosis, such as dynamin and endophilin 2/3, leading to internalization of surface AMPA-type glutamate receptors (AMPARs) (Chowdhury et al., 2006; Rial Verde et al., 2006; Shepherd et al., 2006; Bramham et al., 2008; Waung et al., 2008; Bramham et al., 2010). An increased level of glucocorticoids induced by stress may lead to synaptic plasticity through AMPA receptor trafficking and may in this way promote the consolidation of behaviorally relevant information (Krugers et al., 2010).

Although social defeat induces marked regional specific changes in BDNF and Arc both during adolescence and adulthood, the functional consequences of these changes are far from clear. For example, it is not clear whether social defeat induced increases in Arc mRNA expression lead to LTP or LTD. Several researchers showed that social defeat leads to impaired LTP and to enhanced LTD (Kole et al., 2004; Artola et al., 2006).

Whereas LTP is involved in strengthening of synapses, LTD is followed by low levels of postsynaptic depolarization. LTD counteracts the consequences of LTP by selectively weakening synapses. This is necessary because, if synapses are allowed to continuously increase in strength, synapses would ultimately reach a ceiling level of efficiency (Teyler et al., 1995; Massey and Bashir, 2007; Kessels and Malinow, 2009). Also, the behavioral effects of a change in LTP and LTD after social defeat and the exact role of increased levels of Arc and BDNF are not yet understood.

The behavioral consequences can for example be studied by local knockdown of BDNF (Berton et al., 2006), by scavenging BDNF with the recombinant TrkB-Fc inhibitor (Rex et al., 2007) or by interference with Arc expression by using Arc antisense (Messaoudi et al., 2007).

Taken together, changes in the BDNF pathway induced by social defeat may play an important role in the long lasting behavioral effects of social stress, but are likely to be accompanied by plasticity changes in other signaling pathways as well.

VALIDITY OF THE ANIMAL MODEL

While basic behavioral neuroscience research is mostly performed to understand how the brain controls complex behavior, this is often aimed at generating knowledge to improve human health and welfare. Hence, one may wonder to what extent the current research may contribute to these ultimate goals.

The validity of animal models can be judged by three different criteria, namely face validity, construct validity and predictive validity (McKinney and Bunney, 1969; Willner, 1984; Van der Staay, 2006). We wondered whether the social defeat model and the behavioral measures used in the current thesis are valid models for social stress and its consequences experienced by humans.

Face validity means that a model resembles symptomatology of human disorders (Van

der Staay, 2006). The social defeat models has been shown to induce long-term consequences on behavior which are likely modeling aspects of depressive like disorder in humans (Koolhaas et al., 1997; Ruis et al., 1999), such as disturbed sleep patterns, activity levels (Meerlo et al., 1996b; Meerlo et al., 1996a) and generalized anxiety (Vidal et al., 2007; Vidal et al., 2011c). One should keep in mind that it is difficult to model depression in animals due to the heterogeneity of symptoms of depression. The criteria for neuropsychiatric disorders in humans are based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (2000). Diagnoses of disease are solely based on symptoms, signs and the course of illness and not on objectively measurable abnormalities of molecules, synapses, cells or neural circuits. Only a subset of symptoms (anhedonia, homeostatic symptoms and behavior) can be measured in animals, whereas cognitive symptoms cannot be objectively measured (Nestler and Hyman, 2010). Furthermore, the consequences of social stress are generally seen in the light of internalizing disorders whereas the possibility that social stress leads to externalizing disorders is often neglected.

In **chapter 6**, I showed that social defeat in adolescence does not lead to an overall change in aggression and impulsivity levels in WTG rats. Similarly in **chapter 7**, I showed that only RHA rats show a change in the adult behavioral profile after adolescent social stress. The data of **chapter 8** also show that not all individual show an increase in the expression of synaptic plasticity related genes after social defeat. The fact that not all animals subjected to social defeat show behavioral and neurobiological disturbances contributes to the validity of the model, since in humans large differences in susceptibility to depression are also present.

Construct validity means that the theoretical rationale and the etiology of a model match with the human situation. Stress in humans is often of a psychological nature, however most animal models of stress bear little or no relationship to the situations that animals encounter in daily life and do not involve a social component. Three social stressors that are widely used nowadays are social isolation (Fone and Porkess, 2008), crowding (Brown and Grunberg, 1995) and social defeat (Koolhaas et al., 1997; Huhman, 2006). In the experiments described in this thesis the social defeat model was used, which is a relatively naturalistic model. The resident-intruder paradigm makes use of the natural defense mechanism of animals and induces loss of environmental control in the intruder animal (Koolhaas et al., 1997). By using a social stressor, the experiments described in this thesis have high construct validity. Furthermore, the rationale for the experiments described in this thesis is that the long-term effects induced by social defeat are (partly) mediated by disturbed prefrontal cortex functioning, which is partly based on studies in humans.

The assumption that the prefrontal cortex is maturing during adolescence in both humans and rodents should be taken with caution, since brain maturation has been shown to follow different developmental time patterns in different species. The rodent hippocampus, for example, develops well into adolescence (Meyer et al., 1978), whereas the human hippocampus is fully developed by 2 years of age (Lupien et al., 2009).

The use of rats of the Roman selection lines increased the reproducibility and predictability of the results. A downside of using these selection lines is that we did not use an unselected control line and therefore we cannot completely distinguish the responses in

the two lines from responses induced by, for example, genetic drift. In addition, the Roman rats have been originally selected for differences in active avoidance behavior (Bignami, 1965), which is a relatively unnatural situation. By using rats of the wild-type Groningen strain we made use of the naturally occurring individual variation in the display of aggressive behavior.

High predictive validity of a model means that a model responds to treatments in a way that predicts the effects of those treatments in humans. In the experiments described in this thesis, we did not explore the effects of pharmacological manipulations on brain and behavior. Other studies show that the social defeat model has some predictive validity based on the response to chronic antidepressant treatment. The effects of chronic antidepressant treatment of animals subjected to social defeat are similar to the effects obtained in humans (Von Frijtag et al., 2002; Krishnan et al., 2007).

All experimental subjects in the current thesis were males. Inevitably, the question arises whether the consequences of social stress are gender specific. Studies in human show that the incidence rates for the development of depression during adolescence are higher in females than in males (Hankin et al., 1998; Wade et al., 2002; Bouma et al., 2008). In general, women appear to be more susceptible to stress-related disorders such as depression than men (Blanchard et al., 1995). It is hypothesized that stress in women leads to internalizing disorders, whereas men show more externalizing psychopathological disorders (Kessler et al., 1993; Kessler et al., 1994; Nolen-Hoeksema, 2012). By using aggression and impulsivity as behavioral read-outs or externalizing behaviors in our studies, we used parameters that are mostly relevant for male subjects, but less suitable for the use of females.

The absence of offensive aggression in females is also the major reason for the lack of studies on social defeat in females, since it is difficult to induce strong dominance relationships or highly aggressive residents in females. Most social stress models that are efficient in males are inefficient in females (Haller et al., 1999). However, some studies involve social isolation or chronic mild stress and show gender differences in the response to those stressors. In a study by Bourke and Neigh, chronic mixed modality stress (isolation, restraint and social defeat) during adolescence lead to a differential effect in depressive-like behavior in adult male and female Wistar rats. Female rats showed depressive-like behavior, whereas males exposed to the same stressors were unaffected (Bourke and Neigh, 2011). Other studies show that the consequences of stress during adolescence are gender specific as well (McCormick et al., 2007; Weintraub et al., 2010). One of the few studies on sexual differences in the neurobiological consequences of social stress shows that isolation reduces BDNF mRNA expression in the hippocampus of females, but not of males (Weintraub et al., 2010).

In summary, the animal models used in this thesis exhibit features of face, construct and predictive validity. Experimental research on the consequences of social stress in females would be valuable and would add to the validity of the animal models for study of the development of human psychiatric diseases.

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Nederlandse samenvatting

INLEIDING

Affectieve stoornissen hebben een grote invloed op het leven van miljoenen mensen wereldwijd. Het is bekend dat vroege negatieve ervaringen een voorspeller zijn voor de ontwikkeling van psychopathologie later in het leven. Onderzoek in de afgelopen jaren heeft zich vooral gericht op de ontwikkeling van internaliserende stoornissen zoals depressie en angststoornissen. Deze stoornissen komen met name voor bij vrouwen. Blootstelling aan sociale stress kan echter ook leiden tot externaliserende stoornissen, zoals een antisociale persoonlijkheidsstoornis en de expressie van ongepast en excessief agressief gedrag. Deze zogenaamde externaliserende stoornissen komen meer voor bij mannen.

Agressief gedrag is een van de belangrijkste citeria van externaliserende stoornissen. Vanuit een biologisch perspectief is agressie echter een zeer functionele vorm van sociaal gedrag. Het is betrokken bij competitie voor voedsel, bescherming van het individu en de nakomelingen. Bij de meeste individuen is agressie onderhevig aan sterke remmende invloeden die bijvoorbeeld voorkomen dat agressie overgaat in geweld. Er is sprake van geweld wanneer de expressie van agressie niet onder controle is. Geweld is een groot volksgezondheidsprobleem en heeft aanzienlijke economische gevolgen. Ondanks de grote gevolgen van geweld is het belangrijk om te realiseren dat het merendeel van humane sociale interacties niet escaleren tot geweld. Attentie problemen en impulsiviteit zijn persoonlijkheidseigenschappen die kunnen leiden tot de transitie van agressie in geweld. Deze eigenschappen zijn gerelateerd aan een gebrekkig werkende prefrontale cortex, het hersengebied waarvan wordt aangenomen dat het gedrag inhibeert.

Tijdens de adolescentie ondergaat de prefrontale cortex grote structurele en functionele veranderingen. De ontwikkeling van de prefrontale cortex gaat gepaard met eigenschappen die karakteristiek zijn voor de adolescentie zoals verminderde impuls controle en risico gedrag. Dit uit zich bijvoorbeeld in een verhoogd drugs gebruik. Bovendien is de adolescentie een periode waarin relaties veranderen en veel sociale vaardigheden worden geleerd. De verandering in sociale omgeving en de constante blootstelling aan nieuwe situaties en emoties tijdens de adolescentie kan leiden tot de ervaring van stress. Verstoring van de ontwikkeling van de prefrontale cortex door blootstelling aan stress tijdens de adolescentie zou een sleutelrol kunnen spelen in de ontwikkeling van gewelddadig gedrag.

Het doel van dit proefschrift was om de individuele verschillen in de ontwikkeling van volwassen externaliserende stoornissen (met name agressie en impulsiviteit) als gevolg van de blootstelling aan sociale stress tijdens de adolescentie te bestuderen en de hypothese te testen dat het waargenomen gedrag wordt gemedieerd door veranderingen in synaptische plasticiteit in de prefrontale cortex.

AGRESSIE EN IMPULSIVITEIT

Om de gevolgen van sociale stress tijdens de adolescentie op agressie en impulsiviteit en de rol van synaptische plasticiteit te bestuderen heb ik gebruik gemaakt van een aantal diermodellen. Het zogenaamde ‘resident-intruder’ model werd zowel gebruikt voor het

teweegbrengen van sociale stress als voor het meten van agressief gedrag. Dit model maakt gebruik van het feit dat een dier, dat zijn eigen territorium heeft, dit zal verdedigen. In het geval van het veroorzaken van sociale stress is het experimentele dier de indringer welke wordt aangevallen door de resident. Bij het meten van agressief gedrag wordt juist gekeken naar het gedrag van de resident.

Om individuele verschillende in de vatbaarheid voor stress te bestuderen heb ik voor de experimenten beschreven in dit proefschrift gebruik gemaakt van wild-type Groningen ratten en ratten van de Roman selectielijnen. Wild-type Groningen ratten worden gekenmerkt door grote individuele verschillen in offensief gedrag. Sommige dieren zijn helemaal niet agressief (reactieve dieren), terwijl andere dieren van dezelfde stam zeer agressief (proactief) zijn. Ratten van de Roman selectielijnen zijn selectief gefokt voor het al dan niet actief vermijden van een elektrische schok in een zogenaamde shuttlebox. Hiermee worden twee extreme persoonlijkheden verkregen. Roman high avoidance (RHA) ratten vermijden schokken en zijn proactief, terwijl Roman low avoidance (RLA) ratten een reactief gedragspatroon vertonen.

Verschillende experimenten door anderen hebben laten zien dat de individuele variatie in agressie niveau en gedrag in een shuttlebox taak is gerelateerd aan hoe deze dieren in het algemeen omgaan met stressoren en hun omgeving. De consequente manier waarop individuele dieren zich in verschillende situaties gedragen wordt gezien als het equivalent van persoonlijkheden bij mensen.

Door middel van operant conditioneren heb ik verschillen in impulsiviteit, inhibitie en flexibiliteit van gedrag en de relatie met agressief gedrag bestudeerd. Uit de experimenten beschreven in dit proefschrift blijkt dat, in een onvoorspelbare operant conditioneringstaak, proactieve dieren worden gekenmerkt door rigide gedrag, terwijl reactieve dieren flexibel gedrag vertonen. Proactieve en reactieve dieren verschillen niet in efficiëntie in een test die impulsiviteit in een voorspelbare situatie meet. Gewelddadige dieren blijken zich daarentegen juist te onderscheiden in een dergelijke testsituatie en zijn impulsiever dan 'normaal' agressieve dieren.

Tijdens de verschillende testen voor impulsiviteit werd de cardiovasculaire respons van dieren gemeten met behulp van een bloeddruk en hartslagzender. De resultaten laten zien dat de cardiovasculaire respons de activiteit van het dier reflecteert. De hoogte van de respons is niet gerelateerd aan de persoonlijkheid van dieren.

In dit proefschrift heb ik de hypothese beschreven dat de correlatie van verschillende gedragingen verklaart dient te worden aan de hand van het onderliggende neurobiologische substraat. Wanneer gedragingen gecorreleerd zijn ligt het voor de hand dat hetzelfde hersengebied bij deze gedragingen betrokken is. Op basis van deze hypothese ligt het voor de hand dat RHA ratten door hoge en RLA ratten door lage agressie niveaus worden gekenmerkt. Echter, ondanks dat er grote verschillen zijn in het niveau van offensief gedrag in Roman ratten, is dit niet gerelateerd aan hun gedrag in een shuttlebox test. Een mogelijke verklaring hiervoor is dan ook dat een verschillend neurobiologisch substraat betrokken is bij agressief en shuttlebox gedrag.

Over het algemeen wordt aangenomen dat persoonlijkheid op volwassen leeftijd stabiel is over tijd en in verschillende contexten. Het blijft dan ook de vraag wanneer

persoonlijkheidseigenschappen in de loop van de ontwikkeling ontstaan en wanneer deze eigenschappen zich stabiliseren. Het gedrag in een onvoorspelbare operant conditioneringstaak tijdens de adolescentie blijkt bijvoorbeeld geen voorspeller te zijn voor de persoonlijkheid van volwassen dieren. Omdat er sterke sociale zowel als biologische veranderingen plaatsvinden tijdens de adolescentie is het waarschijnlijk dat stabilisatie van persoonlijkheid pas na deze fase plaats vindt.

VATBAARHEID OF VEERKRACHTIGHEID NA SOCIAAL VERLIES?

De adolescente fase van het leven wordt gekarakteriseerd door grote neurobiologische veranderingen. De prefrontale cortex in het bijzonder ontwikkelt zich nog in deze levensfase. Omdat agressie en impulsief gedrag sterk gereguleerd worden door de prefrontale cortex was de verwachting dat verstoring van de ontwikkeling tijdens de adolescentie langdurige gevolgen kan hebben voor het vertonen van agressief en impulsief gedrag.

Gebleken is dat sociaal verlies tijdens de adolescentie inderdaad leidt tot een verstoring van de relatie tussen agressie en flexibiliteit van gedrag in wild-type Groningen ratten. Omdat de persoonlijkheid van deze dieren niet vooraf te bepalen is, is het moeilijk te zeggen in welke dieren en welke veranderingen precies teweeg worden gebracht.

De persoonlijkheid van ratten van de Roman selectielijnen is al bij de geboorte bekend en het gebruik van deze dieren leidt dan ook tot een duidelijker beeld welke karaktereigenschappen veranderen door sociaal verlies tijdens de adolescentie. Na adolescent sociaal verlies vertonen RHA ratten minder rigide gedrag terwijl het gedrag van RLA ratten niet wijzigt. Er zijn indicaties gevonden dat het agressie niveau wordt verlaagd na social verlies. Of de veranderingen in wild-type Groningen ratten en RHA ratten na sociaal verlies gerelateerd zijn aan een verandering in het functioneren van de prefrontale cortex is niet bewezen.

Sociale stress in adolescente en volwassen dieren leidt in de prefrontale cortex tot een verhoging van de expressie genen die door BDNF (Brain-derived neurotrophic factor) gereguleerd worden. Ook in de hippocampus van jonge dieren, maar niet in de hippocampus van volwassen dieren, wordt een verhoging van BDNF gevonden na sociaal verlies. BDNF is een neurotrofine die neurotransmissie op het niveau van synapsen en plasticiteit van synapsen reguleert. Een verhoging van BDNF expressie wordt door anderen ook gevonden na het toedienen van antidepressiva en heeft mogelijk een beschermend effect voor de gevolgen van stressoren. De hogere expressie in jonge dieren zou dus juist kunnen betekenen dat adolescenten veerkrachtiger zijn dan volwassenen.

De studies beschreven in dit proefschrift laten zien dat sociaal verlies leidt tot een verstoring van de ontwikkeling van agressief en impulsief gedrag en een verandering in synaptische plasticiteit in de prefrontale cortex. Verder onderzoek is nodig om aan te tonen of er een causaal verband bestaat tussen de gevonden veranderingen in synaptische plasticiteit in prefrontale cortex en de gedragsmatige veranderingen.

De vraag of een stressvolle gebeurtenis tijdens de adolescentie individuen vatbaarder of juist weerbaarder maken voor het ontwikkelen van psychopathologie hangt sterk af van

de persoonlijkheid en de omstandigheden waaraan het individu op volwassen leeftijd wordt blootgesteld. Ik heb laten zien dat stressvolle gebeurtenissen tijdens de adolescentie sommige individuen meer vatbaar maken voor psychopathologie, terwijl anderen het juist beter doen onder stressvolle condities na een eerdere ervaring met sociale stress. Het is van belang om in de toekomst niet alleen naar psychopathologie als gevolg van stress te kijken, maar juist ook naar welke individuen veerkrachtig zijn en waarom.

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Coppens CM, Coolen A, de Boer SF, Koolhaas JM. Adolescent social defeat disturbs adult aggression-related impulsivity in wild-type rats (submitted).

CURRICULUM VITAE

Caroline Coppens was born on November 16, 1984 in Alkmaar, the Netherlands. She attended six years gymnasium at C.S.G. Jan Arentsz in Alkmaar and graduated in 2003. She did the bachelor Life Science & Technology (2003-2005) and the master Biomedical science between 2005 and 2008 at the University of Groningen. Her first master research project was performed at the department of behavioral physiology under the supervision of Dr. B. Buwalda where she studied the influence of chemotherapy combined with administration of IGF-1 on behavior and neurogenesis in rats. The second research project was a multi-disciplinary project at the department of synthetic organic chemistry and the department of membrane cell biology in Groningen. She was supervised by Prof. Dr. B. Feringa and Dr. I. Zuhorn. The title of this project was 'Synthesis and analysis of pentaenecationic lipids for the study of lipoplex-mediated gene delivery using fluorescence microscopy'. For her third research she moved to Londen, Ontario in Canada where she worked at the department of Anatomy and Cell Biology at the University of Western Ontario. In this research project, supervised by Prof. Dr. L.M. Coolen, she studied the effects of sexual experience on neuroplasticity in the mesolimbic dopamine system in rats. She graduated *cum laude* in 2008 and started her PhD project at the department of Behavioral Physiology at the University of Groningen under the supervision of Prof. Dr. J.M. Koolhaas and Dr. S.F. de Boer. The work she performed there is shown in this thesis.